

From: [Yingling, Gary L.](#)
To: [McMahon, Carrie](#)
Cc: [Vaughn, Jessica L.](#)
Subject: FW: Impossible Foods Letter Regarding Color Additive Regulations
Date: Wednesday, October 18, 2017 4:04:38 PM
Attachments: [\(93593290\) \(8\) FDA Response Letter - Soy Leghemoglobin and Color Additive Regulations \(2\).DOCX](#)
[Attachment 1 Impossible Foods Patent US9700067.pdf](#)

Dear Dr. McMahon: Attached is a letter to the Agency responding to the color additive question. Please accept my apology because it was suppose to have been sent last week. gary

Gary L. Yingling
Morgan, Lewis & Bockius LLP



From: Vaughn, Jessica L.
Sent: Wednesday, October 18, 2017 3:32 PM
To: Yingling, Gary L.
Subject: Impossible Foods Letter Regarding Color Additive Regulations

Hi Gary,
Letter and Patent attached.

-Jessica

Jessica L. Vaughn, Ph.D.
Regulatory Scientist
Morgan, Lewis & Bockius LLP



DISCLAIMER

This e-mail message is intended only for the personal use of the recipient(s) named above. This message may be an attorney-client communication and as such privileged and confidential and/or it may include attorney work product. If you are not an intended recipient, you may not review, copy or distribute this message. If you have received this communication in error, please notify us immediately by e-mail and delete the original message.

Morgan Lewis

Gary L. Yingling

Senior Counsel

October 18, 2017

Carrie McMahon, Ph.D.
Consumer Safety Officer
Office of Food Additive Safety
U.S. Food and Drug Administration
5001 Campus Drive
College Park, MD 20740

Re: Soy Leghemoglobin Protein as a Flavor Catalyst

Dear Dr. McMahon:

This letter responds to the Food and Drug Administration's ("FDA" or "Agency") color additive inquiry in a September 5, 2017 email to our client, Impossible Foods Inc. ("Impossible Foods"), concerning the use of soy leghemoglobin protein derived from *Pichia pastoris* ("soy leghemoglobin") in the Impossible Burger.

Soy leghemoglobin was originally sourced from soybean root nodules, and is produced by Impossible Foods via fermentation using *Pichia pastoris* that has been genetically modified to express soy leghemoglobin. Impossible Foods uses soy leghemoglobin as an ingredient of the Impossible Burger for the sole purpose of driving the chemistry that creates the flavor and aroma of meat. Impossible spent years researching how raw meat transforms from its bland, metallic taste into the complex profile of flavors and aromas that make cooked meat uniquely appealing and flavorful. Impossible Foods discovered that, when exposed to heat (i.e., cooking) a heme protein present in meat (myoglobin) catalyzes the various vitamins, minerals, amino acids and simple sugars found naturally in the surrounding meat tissues, thereby generating meat flavors and aromas. To create a plant-based analog to meat, Impossible Foods researched various plant-based heme proteins, and chose soy leghemoglobin as it actually performs the same function – as a flavor catalyst - as myoglobin in meat, and in some ways, performs even better than myoglobin (e.g., shelf life). The flavor chemistry made possible by soy leghemoglobin is what makes the plant-based Impossible Burger unique.

Because the flavor chemistry made possible by using soy leghemoglobin is so important and novel, Impossible Foods sought a patent covering the use of soy leghemoglobin as a

Morgan, Lewis & Bockius LLP

1111 Pennsylvania Avenue, NW
Washington, DC 20004
United States

T +1.202.739.3000
F +1.202.739.3001

flavor catalyst in plant-based foods. The Patent and Trademark Office recognized the inventiveness of Impossible Foods's use of soy leghemoglobin to drive flavor chemistry, and on July 11, 2017, granted to Impossible Foods U.S. Patent No. 9,700,067 B2, "Methods and Compositions for Affecting the Flavor and Aroma Profiles of Consumables") (Attachment 1). The patent discusses, at length, the use of the soy leghemoglobin to "modulate the flavor and/or aroma profile of other foods" and provides over 14 examples of various flavor profiles enabled by soy leghemoglobin (*see* pp. 21-34 of Attachment 1). Further supporting soy leghemoglobin's use solely for flavor, like many flavors, its use level in food is limited because it will impart an increasingly strong and disagreeable taste if used at levels above 0.8%.

The Agency noted that in posted material Impossible had referenced the red color of soy leghemoglobin, and the Agency asked whether the soy leghemoglobin was a color additive. Impossible does not believe the leghemoglobin is a color additive and consistent with this point, has removed all references in its materials to this inaccurate characterization of use of soy leghemoglobin. Impossible Foods provides further support of its conclusion through the following reasons.

The definition of the term "color additive" found in 21 U.S.C. § 321(t)(B) requires a material to be "added or applied to a food." Soy leghemoglobin is neither added nor applied to a food; rather, soy leghemoglobin is the most important functional ingredient of the food itself. The burger's other main ingredients are wheat protein, deflavored coconut oil and potato protein; the burger would taste entirely bland in the absence of the meaty flavor and aroma properties made possible by the soy leghemoglobin food ingredient. Without soy leghemoglobin, the Impossible Burger would be no different from any of the legion of "veggie burgers" on the market that may taste good, but would never be mistaken for meat. The flavor function of soy leghemoglobin is so important and integral that, as mentioned above, Impossible has secured its exclusive use by obtaining US Patent No. 9,700,067 B2.

Further, in the color additive regulations, 21 C.F.R. § 70.3(f), a food ingredient such as cherries, green or red peppers, chocolate and orange juice, which contribute their own natural color when mixed with other foods, are not regarded as color additives. Likewise, soy leghemoglobin is a food ingredient used for the sole purpose of flavor that will nonetheless impart its natural color to the food. Soy leghemoglobin drives the flavor chemistry of the burger, and is a critically necessary ingredient for that reason alone.

The food color additive ingredients listed in 21 C.F.R. § 73.1 to § 73.615, have a color property and limited, if any, impact other than color. That is not the case with soy leghemoglobin. Soy leghemoglobin has a critical impact by driving the chemistry that produces the distinctive flavor and aroma of meat. Impossible Foods uses soy leghemoglobin as a flavor catalyst. The heme molecule catalyzes flavor and has a natural color and it is not possible to extract the color from soy leghemoglobin without destroying the flavor properties.


October 18, 2017

Page 3

Thus, as stated at the outset, soy leghemoglobin is used for its ability to impart the flavor and aroma of meat in a plant-based product. It is not a color additive, pursuant to 21 C.F.R. § 70.3(f).

We hope we have addressed your questions.

Sincerely,

A handwritten signature in black ink, which appears to read "Gary L. Yingling". The signature is written in a cursive style. A thick black horizontal bar is drawn across the middle of the signature, obscuring the middle portion of the name.

Gary L. Yingling

GLY



US009700067B2

(12) **United States Patent**
Fraser et al.

(10) **Patent No.:** **US 9,700,067 B2**
(45) **Date of Patent:** **Jul. 11, 2017**

(54) **METHODS AND COMPOSITIONS FOR AFFECTING THE FLAVOR AND AROMA PROFILE OF CONSUMABLES**

(71) Applicant: **Impossible Foods Inc.**, Redwood City, CA (US)

(72) Inventors: **Rachel Fraser**, San Francisco, CA (US); **Patrick O'Reilly Brown**, Stanford, CA (US); **Jessica Karr**, San Francisco, CA (US); **Celeste Holz-Schietinger**, East Palo Alto, CA (US); **Elysia Cohn**, Mountain View, CA (US)

(73) Assignee: **Impossible Foods Inc.**, Redwood City, CA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **14/797,006**

(22) Filed: **Jul. 10, 2015**

(65) **Prior Publication Data**

US 2015/0351435 A1 Dec. 10, 2015

Related U.S. Application Data

(63) Continuation of application No. PCT/US2014/011347, filed on Jan. 13, 2014, which (Continued)

(51) **Int. Cl.**
A23J 1/14 (2006.01)
A23J 3/14 (2006.01)
(Continued)

(52) **U.S. Cl.**

CPC . **A23J 3/14** (2013.01); **A23J 1/12** (2013.01);
A23J 1/14 (2013.01); **A23J 3/227** (2013.01);
(Continued)

(58) **Field of Classification Search**

CPC **A23L 1/221**; **A23L 1/231**; **A23L 1/31427**
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

3,658,550 A 4/1972 Hawley
3,693,533 A 9/1972 Liepa
(Continued)

FOREIGN PATENT DOCUMENTS

CN 1252231 A 5/2000
CN 1301811 A 7/2001
(Continued)

OTHER PUBLICATIONS

Jamieson, J., "Iroquois Stew With Beef, Chicken and Pork"—Bon Appétit, Nov. 1995, <http://www.epicurious.com/recipes/food/views/iroquois-stew-with-beef-chicken-and-pork-865>.*

(Continued)

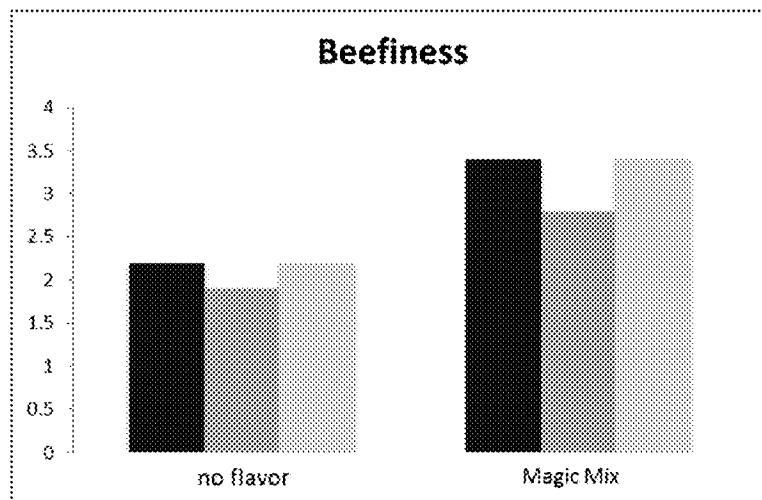
Primary Examiner — Elizabeth Gwartney

(74) *Attorney, Agent, or Firm* — Fish & Richardson P.C.

(57) **ABSTRACT**

This document relates to food products containing highly conjugated heterocyclic rings complexed to an iron ion and one or more flavor precursors, and using such food products to modulate the flavor and/or aroma profile of other foods. The food products described herein can be prepared in various ways and can be formulated to be free of animal products.

23 Claims, 5 Drawing Sheets



Related U.S. Application Data

is a continuation of application No. 13/941,211, filed on Jul. 12, 2013.

- (60) Provisional application No. 61/908,634, filed on Nov. 25, 2013, provisional application No. 61/751,816, filed on Jan. 11, 2013.

(51) **Int. Cl.**

A23J 1/12 (2006.01)
A23J 3/22 (2006.01)
A23L 27/00 (2016.01)
A23L 27/10 (2016.01)
A23L 27/20 (2016.01)
A23L 27/26 (2016.01)
A23L 33/185 (2016.01)
A23L 13/40 (2016.01)

(52) **U.S. Cl.**

CPC *A23L 13/424* (2016.08); *A23L 13/426* (2016.08); *A23L 27/00* (2016.08); *A23L 27/10* (2016.08); *A23L 27/20* (2016.08); *A23L 27/26* (2016.08); *A23L 33/185* (2016.08)

(56) **References Cited**

U.S. PATENT DOCUMENTS

3,829,582 A 8/1974 Guadagni et al.
 3,870,801 A 3/1975 Tombs
 3,966,985 A 6/1976 Jonas
 3,973,043 A 8/1976 Lynn
 4,045,587 A 8/1977 Katz et al.
 4,094,997 A 6/1978 Aishima et al.
 4,218,487 A 8/1980 Jaeggi
 4,435,438 A 3/1984 Lehnhardt et al.
 4,604,290 A 8/1986 Lee et al.
 4,678,676 A 7/1987 Ishizuka et al.
 4,994,285 A 2/1991 Hisano et al.
 5,055,310 A 10/1991 Nonaka et al.
 5,264,239 A 11/1993 Cornet et al.
 5,443,852 A 8/1995 Shahidi et al.
 5,650,554 A 7/1997 Moloney et al.
 5,753,295 A 5/1998 Goldman
 5,807,601 A 9/1998 Carpenter et al.
 5,856,452 A 1/1999 Moloney et al.
 5,922,392 A 7/1999 Kelly et al.
 6,093,424 A 7/2000 Han et al.
 6,146,645 A 11/2000 Deckers et al.
 6,183,762 B1 2/2001 Deckers et al.
 6,210,742 B1 4/2001 Deckers et al.
 6,228,418 B1 5/2001 Gluck
 6,242,036 B1 6/2001 Han et al.
 6,287,620 B1 9/2001 Van Den Ouweland et al.
 6,372,234 B1 4/2002 Deckers et al.
 6,372,961 B1 4/2002 Tarczynski
 6,379,738 B1 4/2002 Dingman et al.
 6,383,531 B1 5/2002 Gottemoller
 6,399,135 B2 6/2002 Gottemoller
 6,413,569 B1 7/2002 Borders et al.
 6,416,797 B1 7/2002 Han et al.
 6,420,148 B2 7/2002 Yamaguchi
 6,495,184 B1 12/2002 Zheng et al.
 6,495,187 B1 12/2002 Borders et al.
 6,509,453 B1 1/2003 Moloney
 6,582,710 B2 6/2003 Deckers et al.
 6,596,287 B2 7/2003 Deckers et al.
 6,599,513 B2 7/2003 Deckers et al.
 6,692,788 B1 2/2004 Mottram et al.
 6,761,914 B2 7/2004 Deckers et al.
 6,908,634 B2 6/2005 Hwang
 6,936,749 B1 8/2005 Guy et al.
 7,052,879 B2 5/2006 Shaw et al.
 7,332,587 B2 2/2008 Moloney
 7,407,786 B2 8/2008 Giver et al.

7,479,472 B1 1/2009 Harbury et al.
 7,585,645 B2 9/2009 Deckers et al.
 7,622,290 B2 11/2009 Brunstedt et al.
 7,666,618 B2 2/2010 Miasnikov et al.
 7,666,628 B2 2/2010 Moloney
 7,674,953 B2 3/2010 Mulet Salort et al.
 7,709,044 B2 5/2010 Ishimoto
 7,807,870 B2 10/2010 Geigenberger et al.
 7,931,925 B2 4/2011 Nielsen
 8,012,732 B2 9/2011 Brunstedt et al.
 8,021,695 B2 9/2011 Gruber et al.
 8,188,415 B2 5/2012 Kats et al.
 8,304,522 B2 11/2012 Kungitani
 8,597,694 B2 12/2013 Guth et al.
 9,011,949 B2 4/2015 Brown et al.
 2001/0024677 A1 9/2001 Bringe
 2001/0049132 A1 12/2001 Kringelum et al.
 2003/0198700 A1 10/2003 Gruber
 2003/0224476 A1 12/2003 Chou
 2004/0151778 A1 8/2004 Richard et al.
 2004/0161513 A1 8/2004 Akashe et al.
 2005/0037111 A1 2/2005 Berry
 2006/0035003 A1 2/2006 McMindes et al.
 2006/0035006 A1 2/2006 McMindes et al.
 2006/0233721 A1 10/2006 Tamarkin et al.
 2007/0269567 A1 11/2007 McMindes et al.
 2007/0269571 A1 11/2007 Akita et al.
 2007/0269583 A1 11/2007 McMindes et al.
 2008/0226810 A1 9/2008 Passe et al.
 2008/0254168 A1 10/2008 Mueller et al.
 2008/0268112 A1 10/2008 Rolan et al.
 2008/0292749 A1 11/2008 Goodwins et al.
 2008/0299254 A1 12/2008 Kim et al.
 2009/0264520 A1 10/2009 Bhagat et al.
 2009/0274817 A1 11/2009 Yamaguchi et al.
 2010/0074998 A1 3/2010 Espeleta Vega et al.
 2010/0136201 A1 6/2010 Bigeard et al.
 2010/0233347 A1 9/2010 Uhrhan
 2010/0249560 A1 9/2010 Levinson et al.
 2010/0281765 A1 11/2010 Schwartz
 2010/0311950 A1 12/2010 Kugitani
 2011/0008502 A1 1/2011 Hosomi et al.
 2011/0064847 A1 3/2011 Miwa et al.
 2011/0064862 A1 3/2011 McCready et al.
 2011/0081386 A1 4/2011 Guth et al.
 2011/0081435 A1 4/2011 Guth et al.
 2011/0117180 A1 5/2011 Yan et al.
 2011/0286992 A1 11/2011 Gruber et al.
 2011/0288389 A9 11/2011 Levinson et al.
 2012/0059150 A1 3/2012 Moloney et al.
 2012/0093994 A1 4/2012 Hsieh et al.
 2013/0004617 A1 1/2013 Zhang et al.
 2015/0296834 A1* 10/2015 Geistlinger A23J 3/04
 426/657
 2015/0296835 A1* 10/2015 Anderson A23J 3/14
 426/106
 2015/0366233 A1 12/2015 Brown et al.

FOREIGN PATENT DOCUMENTS

CN 1407108 A 4/2003
 CN 1466903 1/2004
 CN 1557188 12/2004
 CN 1593223 A 3/2005
 CN 1634524 A 7/2005
 CN 101138405 3/2008
 CN 101541187 A 9/2009
 CN 101606574 A 12/2009
 DE 102007061256 6/2009
 DE 202011002097 3/2011
 EP 0500132 8/1992
 EP 0815736 1/1998
 EP 1166653 1/2002
 EP 1254601 11/2002
 EP 0680751 11/2004
 EP 1529444 5/2005
 EP 1759593 3/2007
 EP 1361264 4/2007
 EP 1952695 8/2008

(56)

References Cited

FOREIGN PATENT DOCUMENTS

EP	2138052	12/2009
GB	2016255	9/1979
JP	52156962	12/1977
JP	S54122766	9/1979
JP	S 573338	1/1982
JP	S5959151	4/1984
JP	2009171877	8/2009
JP	2011000073	1/2011
WO	WO 93/25697	12/1993
WO	WO 94/17673	8/1994
WO	WO 96/17981	6/1996
WO	WO 97/01961	1/1997
WO	WO 98/12913	4/1998
WO	WO 98/53698	12/1998
WO	WO 01/22829	4/2001
WO	WO 01/22830	4/2001
WO	WO 03/070172	8/2003
WO	WO 2004/113543	12/2004
WO	WO 2005/013713	2/2005
WO	WO 2005/097059	10/2005
WO	WO 2006/042608	4/2006
WO	WO 2007/060288	5/2007
WO	WO 2007/115899	10/2007
WO	WO 2007/137125	11/2007
WO	WO 2007/118751	12/2007
WO	WO 2008/017499	2/2008
WO	WO 2008/030089	3/2008
WO	WO 2008/083117	7/2008
WO	WO 2009/060678	5/2009
WO	WO 2010/101625	9/2010
WO	WO 2012/106751	8/2012
WO	WO 2012/110797	8/2012
WO	WO 2012/116703	9/2012
WO	WO 2013/010037	1/2013
WO	WO 2013/010042	1/2013
WO	WO 2013/138793	9/2013

OTHER PUBLICATIONS

Foo, S., "Beef and Scallop Stir Fry"—Food & Wine, Jul. 2001, <http://www.foodandwine.com/recipes/beef-and-scallop-stir-fry>.*

Heller, B., "Barbecued Soybeans"—Vegetarian Soybean recipes, Mother Earth News, Jan./Feb. 1985, <http://motherearthnews.com/real-food/vegetarian-soybean-recipes-zmaz85asie.aspx>.*

Cerny et al., "Formation of Aroma Compounds from Ribose and Cysteine during the Maillard Reaction"—J. Agric. Food Chem., 2003, 51, pp. 2714-2721.*

"Heterologous," Merriam-Webster Dictionary, retrieved on Sep. 10, 2015, <http://www.merriam-webster.com/dictionary/heterologous>, 1 page.

"Silicon Valley gets a taste for food," The Economist Technology Quarterly, Mar. 7, 2015, http://cdn.static-economist.com/sites/default/files/sponsorships/accenture_tq_march2015/20150307_tq_mailout.pdf, pp. 11-13.

"Acidified Milk Products and Protein Stabilisation," Herbstreith & Fox, retrieved on Mar. 3, 2014, <http://www.herbstreith-fox.de/en/informative-literature/informative-literature-from-rd-and-tech-application.html>, 15 pages.

"Ice Cream and Ice Cream Desserts," Herbstreith & Fox, retrieved on Mar. 3, 2014, <http://www.herbstreith-fox.de/en/informative-literature/informative-literature-from-rd-and-tech-application.html>, 5 pages.

"Innovation at Its Best: 5 Years of Food Valley Awards," Food Valley, retrieved on Mar. 7, 2014, <http://www.foodvalley.nl/English/Afbeeldingen/FVAjubileumuitgave/Innovation%20at%20Its%20Best%20-%205%20Years%20of%20Food%20Valley%20Awards.pdf>, 51 pages.

"Low Methylster Amidated Pectins," Herbstreith & Fox, retrieved on Mar. 3, 2014, <http://www.herbstreith-fox.de/en/informative-literature/informative-literature-from-rd-and-tech-application.html>, 13 pages.

"Stabilisation of Whey and Whey Mix Products with Pectin," Herbstreith & Fox, retrieved on Mar. 3, 2014, <http://www.herbstreith-fox.de/en/informative-literature/informative-literature-from-rd-and-tech-application.html>, 6 Pages.

"Texturising of Fermented Milk Products," Herbstreith & Fox, retrieved on Mar. 3, 2014, <http://www.herbstreith-fox.de/en/informative-literature/informative-literature-from-rd-and-tech-application.html>, 6 pages.

Ba et al., "Principles of Meat Aroma flavors and Future Prospect," INTECH Open Science, Open Minds, 2012, Chapter 7, 145-176.

Baek, "Process Flavors," Handbook of Meat, Poultry and Seafood Quality, Second Edition, 2012, Chapter 7, 91-104.

Battaglia et al., "The Enigmatic LEA Proteins and Other Hydrophilins I[W]," Plant Physiology, Sep. 2008, 148:6-24.

Beuchat et al., "Fermentation of Peanut Milk with Lactobacillus bulgaricus and L. acidophilus," J. Food Sci., 1978, 43:1109-1112.

Beyond Better Order page and Nutritional Facts, retrieved on Feb. 6, 2014, <http://www.beyond-better.com/order.html>, 8 pages.

Beyond Meat, posted on or before Feb. 24, 2001, accessed Jan. 7, 2014, <http://beyondmeat.com/>, 2 pages.

Boca Bruschetta Tomato Basil Parmesan Veggie Patties Package Ingredients, posted on or before Jul. 22, 2008, accessed on Jan. 7, 2014, <http://www.bocaburger.com/products/nutrition-info.aspx?product=5928360103>, 1 page.

Boca Flame Grilled Meatless Burgers Package Ingredients, posted on or before Jul. 14, 2008, accessed on Jan. 7, 2014, <http://www.bocaburger.com/products/nutrition-info.aspx?product=5928367321>, 1 page.

Boca Original Meatless Chik'n Nuggets Package Ingredients, posted on or before Jul. 22, 2008, accessed Jan. 7, 2014, <http://www.bocaburger.com/products/nutrition-info.aspx?product=5928360012>, 1 page.

Boca Original Vegan Meatless Burgers Package Ingredients, posted on or before Jul. 14, 2008, accessed Jan. 7, 2014, <http://www.bocaburger.com/products/nutrition-info.aspx?product=5928333445>, 1 page.

Boral and Bohidar, "Effect of Ionic Strength on Surface-Selective Patch Binding-Induced Phase Separation and Coacervation in Similarly Charged Gelatin-Agar Molecular Systems," Journal of Physical Chemistry B, 2010, 114(37): 12027-35.

Bradshaw, "Food 2.0: the future of what we eat," FT Magazine, Oct. 31, 2014, retrieved on Nov. 11, 2014, <http://www.ft.com/cms/s/2/bfa6fca0-5fbb-11e4-8c27-00144feabdc0.html#axzz3InGaCIdL>, 6 pages.

Bute Island Foods, "Sheese," posted on or before Dec. 5, 2006, retrieved on Feb. 6, 2014, http://www.buteisland.com/a_sheese_home.htm, 26 pages.

Chicago Vegan Foods, accessed on Jan. 7, 2014, <http://chicagoveganfoods.com/products/teese-vegan-cheese/>, 8 pages.

Chicago Vegan Foods, Teese Products and Nutrition Facts, posted on or before Mar. 20, 2012, retrieved on Feb. 11, 2014, <http://chicagoveganfoods.com/products/teese-vegan-cheese/>, 10 pages.

Connelly and Piper, "Person of the Year: Tal Ronnen," VegNews, Nov./Dec. 2013, 29-32.

Daiya, Deliciously Dairy Free, "Say Cheese, Dairy-Free cheesy deliciousness," posted on or before Jan. 26, 2010, accessed Jan. 7, 2014, <http://www.daiyafoods.com>, 6 pages.

Davis et al., "Some Rheological Properties of Aqueous Peanut Flour Dispersions," J. Texture Studies, 2007, 38:253-272.

Deliciously Healthy Nacheez, Products and Nutrition Facts, posted on or before Jan. 23, 2011, retrieved on Feb. 7, 2014, <http://nacheez.com/>, 9 pages.

Dixie Diner's Club, Cheese (Not!) Sauce Nutrition Facts, posted on or before Sep. 3, 2009, retrieved on Feb. 7, 2014, <http://www.dixiediner.com/cheese-not-sauce-regular-cheese-p-69.html>, 2 pages.

Door 86 Vegan Cheese, Discover a New World of Vegan Cheese and Menu, posted on or before Dec. 5, 2013, retrieved Feb. 7, 2014, <http://door86vegancheese.wix.com/door-86-vegan-cheese#>, 14 pages.

(56)

References Cited

OTHER PUBLICATIONS

Dr. Cow, Natural Living & Organic Foods, "Aged Cashew & Brazil Nut Cheese," posted on or before Sep. 22, 2008, accessed Feb. 7, 2014, <http://www.dr-cow.com/products/aged-cashew-brazil.html>, 1 page.

Dr. Cow, Natural Living & Organic Foods, "Aged Cashew & Crystal Algae Cheese," posted on or before Sep. 22, 2008, accessed Feb. 7, 2014, <http://www.dr-cow.com/products/aged-cashew-crystal.html>, 1 page.

Dr. Cow, Natural Living & Organic Foods, "Aged Cashew & Dulce Cheese," posted on or before Sep. 22, 2008, accessed Feb. 7, 2014, <http://www.dr-cow.com/products/aged-cashew-dulce.html>, 1 page.

Dr. Cow, Natural Living & Organic Foods, "Aged Cashew & Hemp Seeds Cheese," posted on or before Sep. 22, 2008, accessed Feb. 7, 2014, <http://www.dr-cow.com/products/aged-cashew-hemp.html>, 1 page.

Dr. Cow, Natural Living & Organic Foods, "Aged Cashew Nut Cheese," posted on or before Sep. 22, 2008, accessed Feb. 7, 2014, <http://www.dr-cow.com/products/aged-cashew-nut-cheese.html>, 1 page.

Dr. Cow, Natural Living & Organic Foods, "Aged Macadamia & Hemp Seeds Cheese," posted on or before Sep. 22, 2008, accessed Feb. 7, 2014, <http://www.dr-cow.com/products/aged-macadam-hemp.html>, 1 page.

Dr. Cow, Natural Living & Organic Foods, "Aged Macadamia Nut Cheese," posted on or before Sep. 22, 2008, accessed Feb. 7, 2014, <http://www.dr-cow.com/products/aged-macadam-nut-cheese.html>, 1 page.

Dr. Cow, Natural Living & Organic Foods, "Cashew Nut Cream Cheese," posted on or before Sep. 22, 2008, accessed Jan. 7, 2014, <http://www.dr-cow.com/products/cashew-nut-cream-cheese.html>, 1 page.

Duane, "Engineering the Future of Artisanal Vegan Cheese," Food & Wine, Nov. 2013, <http://www.foodandwine.com/articles/engineering-the-future-of-artisanal-vegan-cheese>, 5 pages.

Ellis et al., "Structure of ferric soybean leghemoglobin a nicotinate at 2.3 Å resolution," *Acta Crystallographica*, May 1997, Section D, 53(3):302-310.

European Search Report (Supplementary) in European Application No. 12810661.4, dated Mar. 12, 2015, 14 pages.

European Search Report (Supplementary) in European Application No. 12811683.7, dated Mar. 12, 2015, 9 pages.

Fantastic World Foods, "Fantastic Foods Nature's Burger (Meatless Burger Mix)," posted on or before Jan. 6, 2009, accessed on Jan. 7, 2014, <http://fantasticfoods.elsstore.com/view/product?id=8715&cid=1967>, 2 pages.

Follow Your Heart Homepage, posted on or before Nov. 28, 1999, accessed Jan. 7, 2014, <http://www.followyourheart.com>, 3 pages.

Follow Your Heart, Products and Nutrition Facts, posted on or before Nov. 28, 1999, accessed Feb. 7, 2014, <http://www.followyourheart.com/products/>, 26 pages.

Foo, "Beef and Scallop Stir-Fry," Food & Wine, Jul. 2001, retrieved on Sep. 10, 2015, <http://www.foodandwine.com/recipes/beef-and-scallop-stir-fry/print>, 3 pages.

Food for Lovers, Vegan Queso Original & Vegan Queso Mild, posted on or before Oct. 27, 2011, retrieved Feb. 7, 2014, <http://www.foodforlovers.com/products>, 3 pages.

Free & Easy Dairy Free Cheese Flavour Sauce Mix, Holland & Barrett, posted on or before Jun. 22, 2013, retrieved Feb. 7, 2014, http://www.hollandandbarrett.com/pages/product_detail.asp?pid=2686, 2 pages.

Fromson, "The Race to Build a Fake-Meat Burger That Just Might Save the World, Free the cows!" *New York Magazine*, Jun. 1-7, 2015, 46-48.

Galaxy Foods Vegan Soy Grated Parmesan, ShopRite, retrieved Feb. 7, 2014, <http://www.shoprte.com/pd/Galaxy-Nutritional-Foods/Vegan-Grated-Soy-Topping-Parmesan-Flavor/4-oz/077172640006/>, 6 pages.

Gardein The Ultimate Beefless Burger Package Ingredients, posted on or before 2013, accessed Jan. 7, 2014, <http://gardein.com/products/beefless-burger/>, 12 pages.

Gardenburger The Original Veggie Burger Package Ingredients, posted on or before Oct. 5, 2008, accessed Jan. 7, 2014, <http://www.gardenburger.com/product.aspx?id=11630>, 1 page.

GenBank Accession No. AFK42304.1, unknown [*Medicago truncatula*], May 25, 2012, 1 page.

Gharst, "Biochemical and Rheological Characteristics of Peanut Proteins Crosslinked with Microbial Transglutaminase," A dissertation submitted to the Graduate Faculty of North Carolina State University, Raleigh NC, 2007, 149 pages.

Gharst, "Effects of Transglutaminase Catalysis on the Functional and Immunoglobulin Binding Properties of Peanut Flour Dispersions Containing Casein," *J. Agric. Food Chem.*, 2008, 56:10913-10921.

Gharst, "The Effect of Transglutaminase Crosslinking on the Rheological Characteristics of Heated Peanut Flour Dispersions," *J. Food Sci.*, 2007, 72(7):C369-C375.

Go Veggie!, "0% Dairy. 100% Yum.," posted on or before 2013, accessed Jan. 7, 2014, <http://goveggiefoods.com/our-products/dairy-free-cheese-alternative-products/>, 1 page.

Gordinier, "Masters of Disguise Among Meatless Burgers," *The New York Times*, Mar. 22, 2011, accessed Jan. 7, 2014, <http://www.nytimes.com/2011/03/23/dining/23meatless.html?pagewanted=all&r=0>, 5 pages.

Hanlon, "Fake Meat: is science fiction on the verge of becoming fact?," *The Guardian*, Jun. 22, 2012, <http://www.theguardian.com/science/2012/jun/22/fake-meat-scientific-breakthroughs-research>, 7 pages.

Heme Protein Database, "Welcome to the Heme Protein Database," posted on or before Apr. 14, 2013, accessed Dec. 18, 2013, <http://hemeprotein.info/heme.php>, 1 page.

Heritage Health Food Creamy Veeta Cheeze Sauce Mix, Vegan Essentials, posted on or before Aug. 13, 2013, retrieved Feb. 7, 2014, <http://store.veganessentials.com/creamy-veeta-cheeze-sauce-mix-by-heritage-health-food-p3945.aspx>, 1 page.

Herper, "Drop that Burger," *Forbes Online*, Nov. 12, 2009, <http://www.forbes.com/forbes/2009/1130/thought-leaders-mcdonalds-global-warming-drop-that-burger.html>, 4 pages.

Homma et al. "Cheese-like food production from various nuts," *Food Preservation Science*, Japan 2009, Abstract.

International Preliminary Report on Patentability in International Application No. PCT/US14/11347, dated Jul. 14, 2015, 10 pages.

International Search Report and Written Opinion in International Application No. PCT/US14/11347, dated Jul. 3, 2014, 20 pages.

Jensen, "Comparative Analysis of Autoxidation of Haemoglobin," *J. Experimental Biology*, 2001, 204:2029-2033.

Ju and Kilara, "Textural Properties of Cold-set Gels Induced from Heat-denatured Whey Protein Isolates," *J. Food Science*, 1998, 63(2): 288-292.

Kanani, "The Future of Meat is Meatless, Just as Tasty, And About to Change the World," *Forbes*, Mar. 6, 2014, retrieved on Sep. 11, 2015, <http://www.forbes.com/sites/rahimkanani/2014/03/06/the-future-of-meat-is-meatless-just-as-tasty-and-about-to-change-the-world/>, 8 pages.

Kraft American Singles Package Ingredients, posted on or before Jun. 27, 2012, accessed on Jan. 7, 2014, <http://www.kraftrecipes.com/Products/ProductInfoDisplay.aspx?SiteId=1&Product=2100060473>, 1 page.

Kung et al., "Tobacco as a Potential Food Source and Smoke Material: Nutritional Evaluation of Tobacco Leaf Protein," *J. Food Sci.*, 1980, 45(2):320-322, 327.

Leahy Gardens Vegan & Delicious, Macaroni & Cheese and Cheese Flavored Sauce Mix Product and Nutrition Facts, posted on or before Feb. 8, 2010, retrieved Feb. 7, 2014, <http://www.leahyfoods.com/products/MacCheese.aspx>, 3 pages.

Lisanatti Foods, Vegan Cheeze Products and Nutrition Facts, posted on or before Mar. 26, 2013, retrieved Feb. 7, 2014, http://www.lisanatti.com/index.php?option=com_zoo&view=category&layout=category&Itemid=22, 5 pages.

(56)

References Cited

OTHER PUBLICATIONS

Liu et al., "Intermolecular Interactions During Complex Coacervation of Pea Protein Isolate and Gum Arabic," *Journal of Agricultural and Food Chemistry*, 2010, 58:552-556.

Lugay and Kim, "Freeze alignment: A novel method for protein texturization," *Utilization of Protein Resources*, 1981, p. 177-187. Luteness, "The Richest Source of Protein," *MOSAIC*, May/Jun. 1979, 39-45.

Maltais et al., "Formation of Soy Protein Isolate Cold-Set Gels: Proteins and Salt Effects," *J. Food Science*, 2005, 70 (1): C67-C73. Morningstar Farms Garden Veggie Patties Package Ingredients, posted on or before Jun. 26, 2013, accessed Jan. 7, 2014, <http://www.morningstarfarms.com/products/burgers/garden-veggie-patties>, 6 pages.

Nacho Mom's Vegan Queso, Products and Nutrition Facts, posted on or before Sep. 20, 2010, retrieved on Feb. 7, 2014, <http://fatgoblin.com/Home.html>, 6 pages.

Nielson, *Introduction to the Chemical Analysis of Foods*, Jones & Bartlett Publishers, 1994.

Nutty Cow Nut Cheeses, Products and Nutrition Facts, posted on or before Jul. 23, 2012, retrieved Feb. 7, 2014, <http://www.nuttycow.com/>, 6 pages.

Parmela Parmesan Style Aged Nut Cheese, Product and Nutrition Facts, 2012, retrieved Feb. 7, 2014, <http://www.parmelafoods.com/your-health.html>, 4 pages.

Peace Cheese 100% Plant-based Cheese Alternative, Product and Nutrition Facts, posted on or before Jun. 6, 2012, retrieved Feb. 7, 2014, <http://www.ilovepeacecheese.com/#/products/4571642621>, 3 pages.

Proulx et al., "Iron Bioavailability of Hemoglobin from Soy Root Nodules Using a Caco-2 Cell Culture Model," *J. Agricultural and Food Chemistry*, Feb. 2006, 54(4):1519-1522.

Punk Rawk Labs: an ongoing experiment in optimal health, Nut Milk Cheese Products, posted on or before Jun. 8, 2011, retrieved Feb. 7, 2014, <http://www.punkrawklabs.net/cheeses.html>, 4 pages.

Reedy et al., "Development of a heme protein structure-electrochemical function database," *Nucleic Acids Research*, 2008, 36:307-313.

Road's End Organics, Cheese Sauce Mix Products and Nutrition Facts, posted on or before Oct. 28, 2009, retrieved Feb. 7, 2014, http://www.edwardandsons.com/reo_shop_chreese.itml, 6 pages.

Road's End Organics, Mac & Chreese Products and Nutrition Facts, posted on or before Oct. 28, 2009, retrieved Feb. 7, 2014, http://www.edwardandsons.com/reo_shop_pastas.itml, 7 pages.

Rusli, "The Secret of These New Veggie Burgers: Plant Blood," *The Wall Street Journal*, Oct. 7, 2014, retrieved on Oct. 9, 2014, <http://online.wsj.com/articles/the-secret-of-these-new-veggie-burgers-plant-blood-1412725267>, 5 pages.

Schwartz, "Meet the Silicon Valley-Backed Vegan Cheese That You Might Actually Eat," *Fast Company*, Feb. 26, 2014, retrieved Sep. 11, 2015, <http://www.fastcoexist.com/3025648/meet-the-silicon-valley-backed-vegan-cheese-that-you-might-actually-eat>, 6 pages.

Sister River Foods Parma!, Products and Nutrition Facts, Posted on or before Jun. 2, 2012, retrieved Feb. 11, 2014, <http://www.veganstore.com/product/parma-vegan-parmesan/vegan-cheese-and-dairy-alternatives>, 6 pages.

Soy Kaas, Products, posted on or before Jan. 20, 2011, retrieved Feb. 11, 2014, <http://www.soykaas.com/products>, 1 page.

Soyco Cheese Products, Natural Pantry, retrieved Feb. 11, 2014, http://www.natural-pantry.com/search_results.asp?ct=All&site_search_qu=soyco&storeID=D92VLAQVMPDL9L5UHTS2WLU67NADEHUA, 10 pages.

Soymage Cheese Products, Good Earth Natural Foods, retrieved on Feb. 11, 2014, http://www.goodearthnaturalfoods.com/shop/brand2.asp?storeID=PJ102JRNHNGT8G0QMPEQ7LDC7GX6C2W2&alpha=S&brand=Soymage&brand_id=805, 6 pages.

Ste Marteen Cheese, Products and Nutrition Facts, posted on or before May 28, 2009, retrieved Feb. 11, 2014, <http://stemarteen.bigcartel.com/>, 14 pages.

The Daiya Advantage, Products and Nutrition Facts, posted on or before Jan. 26, 2010, retrieved on Feb. 7, 2014, <http://us.daiyafoods.com/our-products>, 126 pages.

The Vegetarian Express Parma Zaan Sprinkles, posted on or about Oct. 17, 2009, retrieved Feb. 11, 2014, <http://www.thevegetarianexpress.com/cart/home.php?cat=250>, 2 pages.

Tofu Rella Mozzarella Cheese, Natural Pantry, retrieved Feb. 11, 2014, http://www.natural-pantry.com/shop/product_view.asp?id=24684

&StoreID=D92VLAQVMPDL9L5UHTS2WLU67NADEHUA&private_product=0, 2 pages.

Tofutti Cheese Products and Nutrition, posted on or before Jun. 26, 2013, retrieved Feb. 11, 2014, <http://www.tofutti.com/dairy-free-cheeses/>, 18 pages.

Tofutti Milk Free, "Premium Dairy Free Cheeses," posted on or before Jun. 26, 2013, accessed Jan. 7, 2014, <http://www.tofutti.com/dairy-free-cheeses/>, 2 pages.

Trader Joe's Sliced Soy Cheese Alternative, Fotki, posted Oct. 27, 2008, retrieved Feb. 11, 2014, <http://public.fotki.com/harwons/food/tj-sliced-soy-cheese.html>, 1 pages.

Trader Joe's Vegan Mozzarella, A(soy) Bean, posted Jun. 7, 2013, retrieved Feb. 11, 2014, <http://a-soy-bean.blogspot.com/2013/06/showdown-trader-joes-vegan-mozzarella.html>, 13 pages.

Treeline Treenut Cheese, Products and Nutrition Facts, posted on or before Dec. 10, 2013, retrieved on Feb. 11, 2014, <http://www.treelinecheese.com/treeline-cheese-products.html>, 3 pages.

Van Den Ouweland et al., "Process Meat Flavor Development and the Maillard Reaction," In *Thermal Generation of Aromas*, ACS Symposium Series, American Chemical Society, 1989, 433-441.

VBites, "Cheezly," posted on or before 2013, accessed Jan. 7, 2014, <http://www.vbitesfoods.com/meat-free/cheezly.html>, 2 pages.

Vegan Sun Artisan Aged Raw Cheese, Vegan Essentials, retrieved Feb. 11, 2014, <http://store.veganessentials.com/vegan-sun-artisan-aged-raw-cheese-p4201.aspx>, 3 pages.

VegCuisine Soy Cheese Products, The Vegan Store, retrieved on Feb. 11, 2014, <http://www.veganstore.com/category/s?keyword=vegucuisine>, 5 pages.

Veggie Brothers Mozzarella Sticks, Vegan Essentials, Nov. 9, 2013, retrieved Feb. 11, 2014, <http://store.veganessentials.com/vegan-mozzarella-sticks-by-veggie-brothers-p3761.aspx>, 2 pages.

Victoria Vegan Sauces, Products and Nutrition Facts, posted on or about Sep. 16, 2012, retrieved Feb. 11, 2014, <http://www.victoriafinefoods.com/products/specialty-sauces/victoria-vegan>, 9 pages.

Wayfare We Can't say It's Cheese Spread, Products and Nutrition Facts, posted on or about Oct. 12, 2013, retrieved Feb. 11, 2014, <http://www.wayfarefoods.com/we-cant-say-its-cheese/>, 5 pages.

Wortham and Miller, "Venture Capitalists are Making Bigger Bets on Food Start-Ups," *The New York Times Online*, Apr. 28, 2013, http://www.nytimes.com/2013/04/29/business/venture-capitalists-are-making-bigger-bets-on-food-start-ups.html?pagewanted=all&_r=1&, 4 pages.

Yves Veggie Cuisine The Good Slice, ShopWell, retrieved on Feb. 11, 2014, <http://www.shopwell.com/yves-veggie-cuisine-the-good-slice-cheese-alternative-cheddar-style/soy-foods/p/6082260001>, 1 page.

Clare et al., "Effects of Transglutaminase Catalysis on the Functional and Immunoglobulin Binding Properties of Peanut Flour Dispersions Containing Casein," *J. Agric. Food Chem.*, 2008, 56(22):10913-10921.

Felt, "Raw Vegan Almond Ricotta Cheese," *FeedYourSkull*, Mar. 12, 2012, <https://feedyourskull.com/2012/03/12/raw-vegan-almond-ricotta-cheese/>, 15 pages.

Kummer, "The Problem with Fake Meat," *MIT Technology Review*, Mar. 31, 2015, retrieved Apr. 20, 2016, <<https://www.technologyreview.com/s/536296/the-problem-with-fake-meat/>>, 11 pages.

Sterling, "Welcome to the Era of Plant-Based Meat," *Food & Wine*, Apr. 13, 2016, Retrieved Apr. 20, 2016, <<http://www.foodandwine.com/blogs/welcome-era-plant-based-meat>>, 3 pages.

Aubrey, "Food for Thought: Saving The Planet, One Burger at a Time: This Juicy Patty is Meat-Free," *The Salt*, Feb. 11, 2017, retrieved on Feb. 14, 2017, retrieved from <<http://www.npr.org/>

(56)

References Cited

OTHER PUBLICATIONS

sections/thesalt/2017/02/11/514544431/saving-the-planet-one-burger-at-a-time-this-juicy-patty-is-meat-free>, 14 pages.

"Veggie burgers that look, taste, and bleed like real meat," CBS News, Aug. 9, 2016, retrieved Aug. 25, 2016 <<http://www.cbsnews.com/news/food-trend-veggie-burgers-that-look-bleed-taste-like-real-meat/>>, 4 pages.

"Watch Momofuku Cook Impossible Foods' Plant-Based Burger that 'Bleeds'," Vice, Jul. 27, 2016, retrieved Aug. 25, 2016, <<https://munchies.vice.com/en/videos/watch-momofuku-cook-impossible-foods-plant-based-burger-that-bleeds>>, 3 pages.

Brewer, "The Chemistry of Beef Flavor," Dec. 2006, retrieved on Aug. 30, 2016, <<http://beefresearch.org/CMDocs/BeefResearch/The%20Chemistry%20of%20Beef%20Flavor.pdf>>.

Brooks et al., "Prediction of beef flavor by precursor and volatile compounds Principal Investigators: Funded by The Beef Checkoff," Texas Tech University, May 31, 2012, retrieved Aug. 30, 2016, <http://www.beefresearch.org/CMDocs/BeefResearch/PE_Project_Summaries_FY11Prediction_of_beef_flavor.pdf>.

Calkins et al., "A fresh look at meat flavor," Meat Science, 77(1):63-80 (2007).

Chamlee, "Why Do People Want Veggie Burgers That Bleed?," Eater, Jul. 25, 2016, retrieved Aug. 25, 2016, <<http://www.eater.com/2016/7/25/12270698/lab-grown-meat-beyond-burger-impossible-foods>>, 11 pages.

Dai, "David Chang Adds Plant Based 'Impossible Burger' to Nishi Menu," Jul. 26, 2016, retrieved Jul. 27, 2016 <<http://ny.eater.com/2016/7/26/12277310/david-chang-impossible-burger-nishi>>, 6 pages.

D'Onfro, "I tried the plant-based meat that Google wanted to buy and I never want to eat a 'real' hamburger again" Business Insider, Jun. 12, 2016, retrieved Jun. 14, 2016, <<http://www.businessinsider.com/impossible-burgers-taste-test-2016-6>>, 14 pages.

Donnelly, "Meet the Impossible Burger: It Looks and Tastes Like the Real Thing But is Totally Meat-Free," Vogue, Aug. 1, 2016, retrieved Aug. 25, 2016 <<http://www.vogue.com/13462891/impossible-burger-meat-free-vegan-david-chang>>, 6 pages.

Etienne, "Eating the plant-derived Impossible Burger cooked by Momofuku's David Chang," Tech Crunch, Jul. 26, 2016, retrieved Aug. 25, 2016, <<https://techcrunch.com/2016/07/26/eating-the-plant-derived-impossible-burger-cooked-by-momofuku-david-chang/>>, 9 pages.

European Search Report for International Application No. EP 14737766, dated Jul. 15, 2016, 11 pages.

Gilbert et al., "The revolutionary meatless burger from Impossible Foods is perfect for vegetarians and carnivores alike," Tech Insider, Aug. 4, 2016, retrieved on Aug. 25, 2016, <<http://www.techinsider.io/the-impossible-foods-burger-review-vegetarian-2016-8>>, 9 pages.

Grobart, "Making a Steak Without a Cow," Bloomberg Technology, Jun. 21, 2016, retrieved Jun. 23, 2016 <<http://www.bloomberg.com/news/articles/2016-06-21/making-a-steak-without-the-cow>>, 2 pages.

Herper, "Mission Impossible Burger: Tasting the Fake Meat That Wants to Save the World," Forbes, Jul. 28, 2016, retrieved on Aug. 25, 2016, <<http://www.forbes.com/sites/matthewherper/2016/07/28/mission-impossible-burger-tasting-the-fake-meat-that-wants-to-save-the-world/#57781d823c43>>, 6 pages.

Hoshaw, "Silicon Valley's Bloody Plant Burger Smells, Tastes and Sizzles Like Meat" the salt, Jun. 21, 2016, retrieved Jun. 21, 2016 <<http://www.npr.org/sections/the-salt/2016/06/21/482322571/silicon-valley-s-bloody-plant-burger-smells-tastes-and-sizzles-like-meat>>, 8 pages.

Lopez, "We just tried the 'Impossible Burger'—the meatless burger NYC has been waiting for," Business Insider, Jul. 27, 2016, retrieved on Aug. 25, 2016, <<http://www.businessinsider.com/what-the-impossible-burger-tastes-like-2016-7>>, 5 pages.

Marshall et al., "We Tried the 'Bleeding' Vegetarian Burger and it Was Actually Good," Jul. 27, 2016, retrieved Jul. 28, 2016 <http://www.buzzfeed.com/chelseamarshall/bleeding-vegetable-burger?utm_term=jaa03Kyo7#.ogV0m7MAW>, 10 pages.

Proulx, "Diversified strategies for improving iron bioavailability of maize," Iowa State University—Retrospective Theses and Dissertations, 2007 retrieved on Sep. 19, 2016, retrieved from <<http://lib.dr.iastate.edu/rtd/15852/>>, 144 pages.

Segner, "Meatless burger made possible with local effort," Jul. 29, 2016, retrieved Aug. 1, 2016 <http://www.southernminn.com/owatonna_peoples_press/news/article_3d414149-1040-534d-b1af-bf4f8c486788.html>, 5 pages.

Soller, "The Impossible Burger is Ready for Its (Meatless) Close-Up," The Wall Street Journal, Jun. 14, 2016, retrieved Jun. 21, 2016 <<http://www.wsj.com/articles/the-impossible-burger-is-ready-for-its-meatless-close-up-1465912323>>, 8 pages.

Swanson, "Patenting the Quest for a More Perfect Veggie Burger," JDSUPRA Business Advisor, Jun. 21, 2016, retrieved Jun. 23, 2016 <<http://www.jdsupra.com/legalnews/patenting-the-quest-for-a-more-perfect-72212>>, 13 pages.

Yancey et al., "Effects of total iron, myoglobin, hemoglobin, and lipid oxidation of uncooked muscles on lively flavor development and volatiles of cooked beef steaks," Meat Science, 73:680-686 (2006).

Chau, "Uncanny Patty," The Ringer, Feb. 27, 2017, retrieved on Feb. 28, 2017, retrieved from <<https://theringer.com/impossible-burger-last-meal-on-earth-week-food-f9f14acdb99#.v0cb2hi6e>>, 19 pages.

Lombardi et al., "Total Heme and Non-heme Iron in Raw and Cooked Meats," Journal of Food Science, 67(5):1738-1741 (2002).

Cott et al., "The 'Impossible' Veggie Burger: A Tech Industry Answer to the Big Mac," Business Day, Jan. 13, 2017, retrieved on Jan. 17, 2017, <<https://mobile.nytimes.com/2017/01/13/business/veggie-burger-impossible-burger.html?referer=http://www.drudgereport.com/>>, 7 pages.

Fang et al., "Food Nutrition health theory and technology," China light industry press, p. 448, Jan. 31, 1997 (Chinese Version).

Fourth Chinese Office Action in Chinese Application No. 201280041713.1, dated Nov. 11, 2016, 18 pages (with translation). Supplementary European Search Report for International No. EP 14737909.3, dated Oct. 7, 2016, 10 pages.

Zhengnong et al., "Cihai biological fascicle," Shanghai Lexicographical Publishing House, p. 243, Dec. 31, 1987 (Chinese Version).

Belitz et al., "Aroma Compounds," Food Chemistry, Springer 2009, pp. 340-402.

Belitz et al., Food Chemistry, 3rd revised edition. Springer-Verlag, Berlin (2006), p. 368.

Bunge et al., "Quest Heats up for Alternatives to Beef," The Wall Street Journal, Business News, Nov. 4, 2016, p. B5.

Burdock, "Fenaroli's handbook of flavor ingredients," CRC press, 17 pages (2016).

Cadwallader and Macleod, "16 Instrumental methods for analyzing the flavor of muscle foods," Flavor of Meat, Meat Products and Seafoods, 18 pages (1998).

Chaudhari et al., "The cell biology of taste," 190(3):285-296 (Aug. 2010).

Chen et al., "Effect of Urea on Volatile Generation from Maillard Reaction of Cysteine and Ribose," J. Agric. Food Chem., 48:3512-3516 (2000).

Chen et al., "Influence of Dna on Volatile Generation from Maillard Reaction of Cysteine and Ribose," Nutraceutical Beverages, American Chemical Society, pp. 427-442 (Dec. 2003).

Elmore et al., "Effect of the Polyunsaturated Fatty Acid Composition of Beef Muscle on the Profile of Aroma Volatiles," J. Agric. Food Chem. 47:1619-1625 (1999).

Grigorakis et al., "Organoleptic and volatile aroma compounds comparison of wild and cultured gilthead sea bream (*Sparus aurata*): sensory differences and possible chemical basis," Aquaculture 225:109-119 (2003).

Grosch, "Evaluation of the Key Odorants of Food by Dilution Experiments, Aroma Models and Omission," Chem. Senses 26:533-545 (Jun. 2001).

(56)

References Cited

OTHER PUBLICATIONS

- Hannah, "A fermented feast," Bittersweet, retrieved on Nov. 3, 2016, retrieved from <<https://bittersweetblog.com/2010/06/09/a-fermented-feast/>>, 2 pages.
- Hui et al., "Handbook of meat and meat processing," CRC Press, 2012, retrieved on Dec. 5, 2016, retrieved from <<https://www.crcpress.com/Handbook-of-Meat-and-Meat-Processing-Second-Edition/Hui/p/book/9781439836835>>, 3 pages.
- Jublot et al., "Quantitation of sulphur aroma compounds in maillard model reaction systems of different composition," Expression of Multidisciplinary Flavour Science, 4 pages (2010).
- Karahadian et al., "Action of Tocopherol-Type Compounds in Directing Reactions Forming Flavor Compounds in Autoxidizing Fish Oils," J. Amer. Oil Chem. Soc., 66:1302-8 (1989).
- Kerscher et al., "Quantification of 2-Methyl-3-furathiols, 2-Furfurylthiol, 3-Mercapto-2-pentanone in Heated Meat," J. Agric. Food Chem. 46:1954-1958 (1996).
- Kerth and Miller, "Beef flavor: a review from chemistry to consumer," 25 pages (2015).
- Khan et al., "Meat flavor precursors and factors influencing flavor precursors—A systematic review," Meat Science, 110:278-284 (Dec. 2010).
- Lane et al., "The Variety of Odors Produced in Maillard Model Systems and How They are Influenced by Reaction Conditions," The Maillard Reaction in Foods and Nutrition, American Chemical Society, pp. 141-158 (Apr. 1983).
- Leduc et al., "Differentiation of fresh and frozen/thawed fish, European sea bass (*Dicentrarchus labrax*), gilthead seabream (*Sparus aurata*), cod (*Gadus morhua*) and salmon (*Salmo salar*), using volatile compounds by SPME/GC/MS," J. Sci. Food Agric., 92:2560-80 (2012).
- McGorin, "Advances in Dairy Flavor Chemistry," Food Flavors and Chemistry: Advances of the New Millennium, Spanier, A. M.; Shahidi, F.; Parment, T. H.; Mussinan, C. J.; Ho, C.-T.; Contis, E. T., Eds., Royal Society of Chemistry, Cambridge, pp. 67-84 (2001).
- McGorin, "Character-impact flavor and off-flavor compounds in foods," Flavor, Fragrance, and Odor Analysis, 2nd, 207-262 (2012).
- McGorin, "The significance of volatile sulfur compounds in food flavors," Volatile sulfur compounds in food 1068, 29 pages (2011).
- Moon et al., "Odour-active components of simulated beef flavour analyzed by solid phase microextraction and gas chromatography-mass spectrometry and -olfactometry," Food Research International, 39:294-308 (Apr. 2006).
- Morita, "Comparison of aroma characteristics of 16 fish species by sensory evaluation and gas chromatographic analysis," J. Sci. Food Agric., 83:289-297 (2003).
- Mottram et al., "Formation of Sulfur Aroma Compounds in Reaction Mixtures Containing Cysteine and Three Different Forms of Ribose," J. Agric. Food Chem., 50:4080-4086 (2002).
- Mottram, "Flavour formation in meat and meat products: a review," Food Chemistry, 62(4):415-24 (Aug. 1998).
- Mottram, "An Overview of the Contribution of Sulfur-Containing Compounds to the Aroma in Heated Foods," Heteroatomic Aroma Compounds, American Chemical Society, pp. 73-92 (Aug. 2002).
- Ramos et al., "What is Masa?—Ingredient Intelligence," The Kitchn, retrieved on Dec. 1, 2016, <http://www.thekitchn.com/whats-the-difference-between-masa-and-masa-harina-226434>, 5 pages.
- Richins et al., "Effect of Iron Source on Color and Appearance of Micronutrient-Fortified Corn Flour Tortillas," Cereal Chem., 85:561-5 (2008).
- Rochet and Chaintreau, "Carbonyl Odorants Contributing to the In-Oven Roast Beef Top Note," J. Agric. Food Chem., 53:9578-9585 (Nov. 2005).
- Rowe, "Chemistry and technology of flavors and fragrances," Oxford: Blackwell; 2005, 351 pages.
- Schieberle et al., "Characterization of Key Odorants in Dry-Heated Cysteine-Carbohydrate Mixtures: Comparison with Aqueous Reaction Systems," Flavor Analysis, American Chemical Society, pp. 320-330 (September 1998).
- Selli et al., "Odour-active and off-odour components in rainbow trout (*Oncorhynchus mykiss*) extracts obtained by microwave assisted distillation-solvent extraction," Food Chemistry, 114:317-322 (2009).
- Shahidi et al., "Meat flavor volatiles: A review of the composition, techniques of analysis, and sensory evaluation," CRC Critical Reviews in Food Science and Nutrition, 24(2):141-243 (Jan. 1986).
- Shi et al., "Identification of characteristic flavour precursors from enzymatic hydrolysis-mild thermal oxidation tallow by descriptive sensory analysis and gas chromatography-olfactometry and partial least squares regression," Journal of Chromatography B, 913-914:96-76 (Jan. 2013).
- Shu et al., "Parameter Effects on the Thermal Reaction of Cystine and 2,5-Dimethyl-4-hydroxy-3(2H)-furanone," Thermal Generation of Aromas, American Chemical Society, pp. 229-241 (Oct. 1989).
- Song, et al., "Contribution of oxidized tallow to aroma characteristics of beeflike process flavour assessed by gas chromatography-mass spectrometry and partial least squares regression," Journal of Chromatography A, 1254:115-124 (Sep. 2012).
- Spence et al., "Multisensory Flavor Perception," Cell 161: 24-35 (2015).
- Supplementary Partial European Search Report in European Application No. 14738061 dated Nov. 7, 2016, 11 pages.
- 43.Of Tang et al., "Flavor chemistry of 2-methyl-3-furanthiol, an intense meaty aroma compound," Journal of Sulfur Chemistry, 11 pages, (2012).
- The Good Scents Company, "The Good Scents Company Information System," 2015, retrieved on Dec. 1, 2016, <http://www.thegoodscentscompany.com/>, 2 pages.
- Tressl et al., "Formation of Amino Acid Specific Maillard Products and Their Contribution to Thermally Generated Aromas," Thermal Generation of Aromas, American Chemical Society, pp. 156-171 (Oct. 1989).
- Uauy et al., "Iron Fortification of Foods: Overcoming Technical and Practical Barriers," J. Nutr. 132:849S-852S (2002).
- Withycombe et al., "Identification of 2-Methyl-3-Furanthiol in the Steam Distillate from Canned Tuna. Fish," Journal of Food Science, 53(2):658-660 (1988).
- Fang et al., "Food Nutrition health theory and technology," China light industry press, p. 448, Jan. 31, 1997 (English Translation).
- Zhengnong et al., "Cihai biological fascicle," Shanghai Lexicographical Publishing House, p. 243, Dec. 31, 1987 (English Translation).
- "Rethink Meat," Presented at the 6th Annual Sustainable Innovation Forum at the 2015 United Nations Climate Change Conference (aka COP21), Paris, France, Dec. 7-8, 2015, retrieved on Feb. 1, 2016, <https://amp.twimg.com/v/7c7f7084-b173-42cb-bc12-723f35994dff>, 1 page (Video Submission).
- Asgar et al., "Nonmeat Protein Alternatives as Meat Extenders and Meat Analogs," Comprehensive Reviews in Food Science and Food Safety, 2010, 9:513-529.

* cited by examiner

FIG. 1

SEQ ID NO:1 *Vigna radiata*

MTTTLERGFTEEQEALVVKSWNVMMKNSGELGLKFFLKIFEIAPSAQKLFSFLRDSTVPLEQNPK
LKPHAVSVFVMTCDASVQLRKAGKVTVRESNLKKGATHFRTGVANEHFVTKFALLETIKEAVP
EMWSPAMKNAWGEAYDQLVDAIKYEMKPPSS

SEQ ID NO:2 *Methylobacterium thermophilum*

MIDQKEKELIKESWKRIEPPNKNEIGLLFYANLFKEEPTVSVLFQNPISQSRKLMQVLGILVQGI
DNLEGLIPTLQDLGRRHKQYGVVDSHYPLVGDCLLKSIQEYLGQGFTEEAKAAWTKVYGIAAQVM
TAE

SEQ ID NO:3 *Aquifex aeolicus*

MLSEETIRVIKSTVPLLKEHGTEITARMYELLFSKYPKTKELFAGASEEQPKKLANAIIAYATYI
DRLEELDNAISTARSHVRRNVKPEHYPLVKECLLQAIIEVLNPGEEVLKAWEEAYDFLAKTLIT
LEKKLYSQP

SEQ ID NO:4 *Glycine max*

MGAFTEKQEALVSSSFEAFKANIPQYSVVEYTSILEKAPAAKDLFSFLSNGVDPSNPKLTGHAEK
LFGLVDRDSAGQLKANGTVVADAALGSIHAQKAITDPQFVVVKEALLKTIKEAVGDKWSELSAW
EVAYDELAAAIAKKAF

SEQ ID NO:5 *Hordeum vulgare*

MSAAEGAVVFSEEKEALVLKSWAIMKKDSANLGLRFFLKIFEIAPSAQKLFSLRDSDVPLETNP
KLKTHAVSVFVMTCEAAAQLRKAGKITVRETTLLKRLGGTHLKYGVADGHFEVTRFALLETIKEAL
PADMWGPENRANWGEAYDQLVAAIKQEMKPAE

SEQ ID NO:6 *Magnaporthe oryzae*

MDGAVRLDWTGLDLTGHEIHDGVPIASRVQVMVSFPLFKDQHIIMSSKESPSRKSSTIGQSTRNG
SCQADTQKQGLFPVGEKPKPVKENPMKKLKEMSQRPLPTQHGDTYPTTEKKLTGIGEDLKHIRGY
DVKTLLAMVKSCLKGEKLDKDTMLMERVMQLVARLPTESSKRAELTDSLINELWESLDHPPNLN
LGPEHSYRTPDGSYNHPFNPNQLGAAGSRYARSVIPTVTPPGALPDPLIFDSIMGRTPNSYRKHP
NNVSSILWYATIIHDIPTWTDPRDINTNKSSSYLDLAPLYGNSQEMQDSIRTFKDGMRMKPDCYA
DKRLAGMPPGVSVLLIMFNRFHNVHAENLALINEGGFRNPKPSDLLEGEAREAAWKKYDNDLFQVA
RLVTSGLYINITLVVDYVRNIVNLNRVDTTWTLDPRQDAGAHVGTADGAERGTGNVSAEFNLQCYR
WHSCISEKDSKFVEAQFQNIFFGKPASEVRPDEMWWKGFAMKQNTPADPGQRTFGGFKRGPDGKFD
DDDLVRCISEAVEDVAGAFGARNVPQAMKVETMGIIQGRKWNVAGLNEFRKHHLKPYSTFEDI
NSDPGVAEALRRLYDHPDNVELYPGLVAEEDKQPMVPGVGIAPTYTISRVLSDAVCLVRGDRFY
TTDFTPRNLTNWGYKEVDYDLSVNHGCVFYKLFIRAFPNHFKQNSVYAHYPMVVPSENKRILEAL
GRADLDFEAPKYIPPRVNITSYGAHEYIILETQEKYKVTWHEGLGFLMGEGGLKFMLSGDDPLHA
QQRKCMQAQLYKDGWTEAVKAFYAGMMEELLVSKSYFLGNNKHRHVDIIRDVGNMVHVHFASQVF
GLPLKTAKNPTGVFTEQEMYGILAAIFTTIFFDLDPSKSFPLRTKTREVCQKLAKLVEANVKLIN
KIPWSRGMFVGKPAKDEPLSIYGKTMKGLKAHGLSDYDIAWVHVPTSGAMVNPQAQVFAQAVD
YYLSPAGMHYIPEIHMVALQPSTPETDALLGYAMEGIRLAGTFGSYREAAVDDVVKEDNGRQVP
VKAGDRVFSFVDAARDPKHFPDEPVNPRRPAKKYIHYGVGPHACLGRDASQIAITEMFRCLFR
RRNVRVPVPGQELKKVPRPGGFYVYMRDWWGLFPFPVTMRVMWDDE

SEQ ID NO:7 *Fusarium oxysporum*

MKGSATLAFALVQFSAASQLVWPSKWDEVEDLLYMQGGFNKRGFADALRTCEFGSNVPGTQNTAE
WLRTAFHDAITHDAKAGTGGLDASIYWESSRPENPGKAFNNTFGFFSGFHNPRATASDLTALGTV
LAVGACNGPRIPFRAGRIDAYKAGPAGVPEPSTNLKDTFAAFTKAGFTKEEMTAMVACGHAIGGV

FIG. 1-CONT.

HSVDFPEIVGIKADPNNDTNVPFQKDVSSFHNGIVTEYLAGTSKNPLVASKNATFHSDKRIFDND
KATMKKLSTKAGFNSMCADILTRMIDTVPKSVQLTPVLEAYDVRPYITELSLNNKNKIHFSGSVR
VRITNNIRDNDNLAINLIYVGRDGKKVTVPTQQVTFQGGTSFGAGEVFANFEFDTTMDAKNGITK
FFIQEVKPKSTKATVTHDNQKTGGYKVDDTVLYQLQQSCAVLEKLPNAPLVVTAMVRDARAKDAL
LRVAHKKPVKGSIVPRFQTAITNFKATGKKSSGYTGFGAKTMFEEQSTYFDIVLGGSPASGVQFL
TSQAMPSQCS

SEQ ID NO:8 *Fusarium graminearum*

MASATRQFARAATRATRNGFAIAPRQVIRQQGRRYSSSEPAQKSSSAWIWLTGAAVAGGAGYYFY
GNSASSATAKVFNPSKEDYQKVYNEIAARLEEKDDYDDGSYGPVLVRLAWHASGYDKETGTGGS
NGATMRFAPESDHGANAAGLAAARDFLQPVKEKFPWITYSDLWILAGVCAIQEMLGPAIPYRPGRS
DRDVSGCTPDGRLPDASKRQDHLRGIFGRMGFNDQEI VALSGAHALGRCHTD RSGYSGPWTFSP
VLTNDYFRLLVEEKWQKKWNGPAQYEDKSTKSLMMLPSDIALIEDKKFKPWVEKYAKDNDAFFK
DFSNNVLRLELGVPPFAQGTENQRWTFKPTHQE

SEQ ID NO: 9 *Chlamydomonas eugametos*

MSLFAKLGGREAVEAAVDKFYNKIVADPTVSTYFSNTDMKVQRSKQFAFLAYALGGASEWKGD
RTAHKDLVPHLSDVHFQAVARHLSDTLTELGVPPEDITDAMAVVASTRTEVLNMPQQ

SEQ ID NO:10 *Tetrahymena pyriformis*

MNKPQTIYEKLGGENAMKAAVPLFYKKVLADERVKHFFKNTDMDHQTKQQTDFLTMLLGGPNHYK
GKNMTEAHKGMNLQNLHFDALIIENLAATLKELGVTDAVINEAAKVIEHTRKDMLGK

SEQ ID NO:11 *Paramecium caudatum*

MSLFELQGGQAAVQAVTAQFYANIQADATVATFFNGIDMPNQTNKTA AFLCAALGGPNAWTGRNL
KEVHANMGSNAQFTTVIGHLR SALTGAGVAAALVEQTVAVAETVRGDVVTV

SEQ ID NO:12 *Aspergillus niger*

MPLTPEQIKI I KATVPVLQ EYGT KITTA FYMNMSTVHP ELNAVNTANQVKGHQARALAGALFAY
ASHIDDLGALGPAVELICNKHASLYIQADEYKIVGKYLLEAMKEVLGDACTDDILDAGGAAYWAL
ADIMINREAALYKQSQG

SEQ ID NO:13 *Zea mays*

MALAEADDGAVVFGEEQEALVLKSWAVMKKDAANLGLRFFLKVFEIAPSAEQMF SFLRDSVPLE
KNPKLKTHAMSVFVMTCEAAAQLRKAGKVTVRDTTLKRLGATHLRYGVADGHFEVTGFALLE
EALPADMWSLEMKKAWAEAYSQLVAAIKREMKPDA

SEQ ID NO:14 *Oryza sativa subsp. japonica*

MALVEGNNGVSGGAVSFSEEQEALVLKSWAIMKKDSANIGLRFLLKIFEVAPSASQMF SFLRNSD
VPLEKNPKLKTHAMSVFVMTCEAAAQLRKAGKVTVRDTTLKRLGATHFKYGVGDAHFEVTRFALL
ETIKEAVPVDMMWSPAMKSAWSEAYNQLVAAIKQEMKPAE

SEQ ID NO:15 *Arabidopsis thaliana*

MESEGIKIVFTEEQEALVVKSWSVMKKNSAELGLKLFIKIFEIAPT T KKMFSFLRDSPIPAEQNPK
LKPHAMSVFVMCCESAVQLRKTGKVTVRDTTLKRLGASHSKYGVVDEHFEVAKYALLETIKEAVP
EMWSPKMAVWGQAYDHLVAAIKAEMNLSN

FIG. 1-CONT.

SEQ ID NO:16 *Pisum sativum*

MGFTDKQEALVNSSWESFKQNLSGNSILFYTTIILEKAPAAKGLFSFLKDTAGVEDSPKLQAHAEQ
VFGLVRDSAAQLRTKGEVVLGNATLGAIHVQRGVTDPHFVVVKEALLQTIKKASGNNWSEELNTA
WEVAYDGLATAIKKAMT

SEQ ID NO:17 *Vigna unguiculata*

MVAFSDKQEALVNGAYEAFKANIPKYSVVFYTTILEKAPAAKNLFSFLANGVDATNPKLTGHAEK
LFGLVRDSAAQLRASGGVVADAALGAVHSQKAVNDAQFVVVKEALVKTLKEAVGDKWSDELGTAV
ELAYDELAAAIAKKAY

SEQ ID NO:18 *Bos taurus*

MGLSDGEWQLVLNVAWGKVEADVAGHGQEVLRIRLFTGHPETLEKFDKFKHLKTEAEMKASEDLKKH
GNTVLTALGGILKKKGHHEAEVKHLAESHANKHKIPVKYLEFISDAIIHVLHAKHPSDFGADAQA
AMSKALELFRNDMAAQYKVLGFHG

SEQ ID NO:19 *Sus scrofa*

MGLSDGEWQLVLNVWGKVEADVAGHGQEVLRIRLFKGHPETLEKFDKFKHLKSEDEMKAASEDLKKH
GNTVLTALGGILKKKGHHEAELTPLAQSHATKHKIPVKYLEFISEAIIQVLQSKH
PGDFGADAQGAMSKALELFRNDMAAKYKELGFQG

SEQ ID NO:20 *Equus caballus*

MGLSDGEWQQVLNVWGKVEADIAAGHGQEVLRIRLFTGHPETLEKFDKFKHLKTEAEMKASEDLKKH
GTVVLTALGGILKKKGHHEAELKPLAQSHATKHKIPVKYLEFISDAIIHVLHSKH
PGDFGADAQGAMTKALELFRNDIAAKYKELGFQG

SEQ ID NO:21 *Nicotiana benthamiana*

MSSFTEEQEALVVKSWDSMKKNAGEWGLKFLKIFEIAPSAKKLFSFLKDSNVPLEQNAKLKPHS
KSVFVMTCEAAVQLRKAGKVVRDSTLKKLGATHFYGVADDEHFEVTKFALLETIKEAVPEMWSV
DMKNWGEAFDQLVNAIKTEMK

SEQ ID NO:22 *Bacillus subtilis*

MGQSFNAPYEAIGEELLSQLVDTFYERVASHPLLKPIFPSDLTETARKQKQFLTQYLGGPPLYTE
EHGHPMLRARHLPPFITNERADAWLSCMKDAMDHVGLEGEIREFLFGRLELTARHMVNQTEAEDR
SS

SEQ ID NO:23 *Corynebacterium glutamicum*

MTTSENFYDSVGGEETFSLIVHRFYEQVPNDILGPMYPPDDFEGAEQRLKMFSLSQYWGGPKDYQ
EQRGHPRLRMHRVNYPIGVTAERWLQLMSNALDGVDLTAEQREAIWEHMOVRAADMLINSNPDPH
A

SEQ ID NO:24 *Synechocystis* PCC6803

MSTLYEKLGGTTAVDLAVDKFYERVLQDDRIKHHFFADVDMAKQRAHQKAFLTYAFGGTDKYDGRY
MREAHKELVENHGLNGEHFDAVAEDLLATLKEMGPEDLIAEVAAVAGAPAHKRDVLNQ

SEQ ID NO:25 *Synechococcus* sp. PCC 7335

MDVALLEKSFEQISPRAIIEFSASFYQNLFHHPPELKLPLFAETSQTIQEKKLIFSLAAIENLRNP
DILQPALKSLGARHAEVGTIKSHYPLVGQALIIETFAEYLAADWTEQLATAWVEAYDVIASMTIEG
ADNPAAYLEPELTFYEWLDLYGEESPKVRNAIATLTHFYGEDPQDVQDRSRG

FIG. 1-CONT.

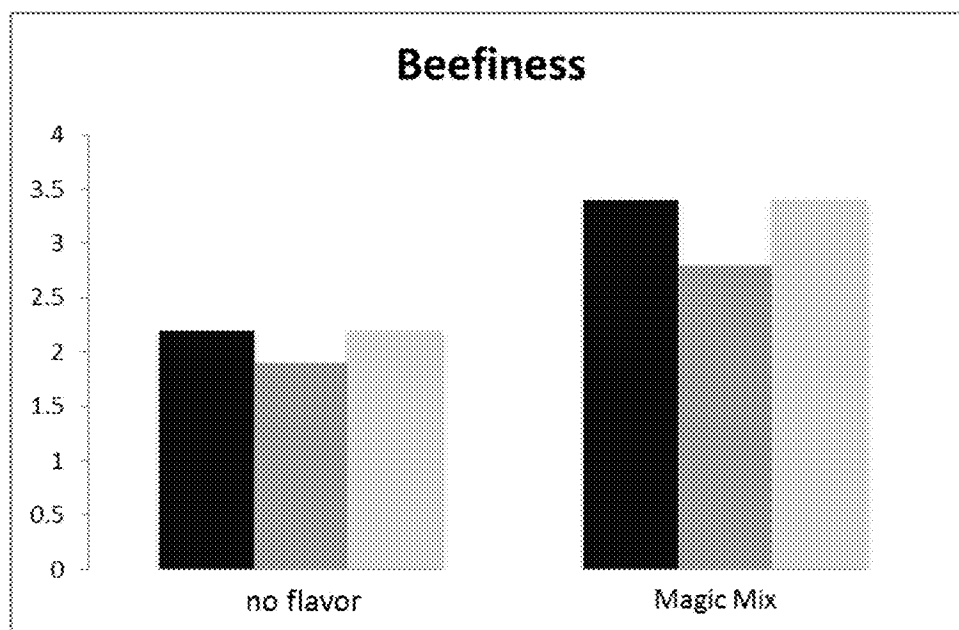
SEQ ID NO:26 *Nostoc commune*

MSTLYDNIGGQPAIEQVVDELHKRIATDSLLAPVFAGTDMVKQRNHLVAFLAQIFEGPKQYGGRP
MDKTHAGLNLQQPHFDAIAKHLGERMAVRGVSAENTKAALDRVVTNMKGAILNK

SEQ ID NO:27 *Bacillus megaterium*

MREKIHSPYELLGGEHTISKLVDAFYTRVGQHP ELAPIFPDNL TETARKQKQFLTQYLGGPSLYT
EEHGH PMLRARHLPFEITPSRAKAWLTCMHEAMDEINLEGPERDELYHRLILTAQHMIN SPEQTD
EKGFSH

FIG. 2



METHODS AND COMPOSITIONS FOR AFFECTING THE FLAVOR AND AROMA PROFILE OF CONSUMABLES

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation and claims priority to PCT/US2014/011347 which claims priority to U.S. application Ser. No. 13/941,211, filed Jul. 12, 2013, U.S. Application Ser. No. 61/908,634, filed Nov. 25, 2013, and to U.S. Application Ser. No. 61/751,816, filed Jan. 11, 2013, and is related to the following patent applications: Application Serial No. PCT/US12/46552; Application Serial No. PCT/US12/46552; Application Ser. No. 61,876,676, filed Sep. 11, 2013; and Application Ser. No. 61/751,818, filed Jan. 11, 2013, all of which are incorporated herein by reference.

TECHNICAL FIELD

This invention relates to food products and more particularly, to food products that include a highly conjugated heterocyclic ring complexed to iron such as a heme-cofactor and one or more flavor precursor molecules.

BACKGROUND

Food is any substance that is either eaten or drunk by any animal, including humans, for nutrition or pleasure. It is usually of plant or animal origin, and can contain essential nutrients, such as carbohydrates, fats, proteins, vitamins, or minerals. The substance is ingested by an organism and assimilated by the organism's cells in an effort to produce energy, maintain life, or stimulate growth.

Food typically has its origin in a photosynthetic organism, such as a plant. Some food is obtained directly from plants, but even animals that are used as food sources are raised by feeding them food which is typically derived from plants.

In most cases, the plant or animal food source is fractionated into a variety of different portions, depending upon the purpose of the food. Often, certain portions of the plant, such as the seeds or fruits, are more highly prized by humans than others and these are selected for human consumption, while other less desirable portions, such as the stalks of grasses, are typically used for feeding animals.

Current plant-based meat substitutes have largely failed to cause a shift to a vegetarian diet. Meat substitute compositions are typically extruded soy/grain mixtures which largely fail to replicate the experience of cooking and eating meat. Common limitations of plant-based meat substitute products are a texture and mouth-feel that are more homogenous than that of equivalent meat products. Furthermore, as these products must largely be sold pre-cooked, with artificial flavors and aromas pre-incorporated, they fail to replicate the aromas, flavors, and other key features, such as texture and mouth-feel, associated with cooking or cooked meat. As a result, these products appeal largely to a limited consumer base that is already committed to vegetarianism/veganism, but have failed to appeal to the larger consumer segment accustomed to eating meat. It would be useful to have improved plant-based meat substitutes which better replicate the aromas and flavors of meat, particularly during and/or after cooking.

SUMMARY

Provided herein are methods and compositions for modulating the flavor and/or aroma profile of consumable food

products, including animal- or non-animal (e.g., plant) based food products, or mixtures of animal- and non-animal-based food products. In some embodiments, the methods and compositions are useful for modulating the flavor and/or aroma profile of a consumable food product during and/or after the cooking process. In some embodiments, the methods and compositions are used to generate one or more chemical compounds that modulate the flavor and/or aroma profile of the consumable food product during and/or after the cooking process.

As provided herein, and without being bound by theory, certain characteristic meaty flavors and/or aromas (e.g., beefy, bacony, umami, savory, bloody, brothy, gravy, metallic, bouillon-like; see Tables 2, 7, and 11), including one or more specific chemical compounds associated with the same (see Tables 3, 8, 9, 12, 14, 16, or 17), are believed to be produced during the cooking process of a consumable food product by chemical reaction of one or more flavor precursor molecules or compositions catalyzed by the presence of a highly conjugated heterocyclic ring complexed to an iron ion (e.g., a heme moiety; or a porphyrin; a porphyrinogen; a corrin; a corrinoid; a chlorin; a bacteriochlorophyll; a corphin; a chlorophyllin; a bacteriochlorin; or an isobacteriochlorin moiety complexed to an iron ion). Such highly conjugated heterocyclic moieties include heterocyclic aromatic rings composed of one or more (2, 3, or 4 more) pyrrole, pyrrole-like, and/or pyrroline subunits. The highly conjugated heterocyclic ring complexed to an iron ion is referred to herein as an iron complex. In some embodiments, the heme moiety can be a heme cofactor such as a heme moiety bound to a protein; a heme moiety bound to a non-proteinaceous polymer; a heme moiety bound to a solid support; or a heme moiety encapsulated in a liposome. In some embodiments, the flavors and/or aromas are not generated in the absence of the iron complex (e.g., in the absence of a ferrous chlorin) or are not generated in the absence of a heme-cofactor (e.g., in the absence of a heme-containing protein). Accordingly, as described herein, the iron complexes such as isolated chlorin-iron complexes or heme-cofactors (e.g., heme-containing proteins) can be used to generate meaty flavors and/or aromas in a variety of food products, such as during the cooking process.

Combining one or more iron complexes such as a heme-cofactor (e.g., a heme-containing protein, including, for example a plant-derived heme protein such as a plant leghemoglobin (legH)), with one or more flavor precursor molecules or compositions (see, e.g., Table 1 or Table 13) can generate or provide a range of savory and meaty aromas and tastes (see, e.g., Tables 2, 7, and/or 11) in a cooked consumable food product. Flavor precursor molecules or compositions can be added to the uncooked food product in purified form and/or can be derived from ingredients in the uncooked consumable food product that contain and/or are enriched with one or more of the particular flavor precursors or compositions, including, for example, yeast extract, vegetable oil, corn oil, soybean oil, palm fruit oil, palm kernel oil, safflower oil, flaxseed oil, rice bran oil, cottonseed oil, olive oil, canola oil, sunflower oil, coconut oil, mango oil, or an algal oil. The resultant flavor and/or aroma profile can be modulated by the type and concentration of the flavor precursors, the pH of the reaction, the length of cooking, the type and amount of iron complex (e.g., a heme cofactor such as a heme-containing protein), the temperature of the reaction, and the amount of water activity in the product, among other factors.

One or more flavor precursor molecules or compositions can be added along with a iron complex (e.g., ferrous

chlorophyllin or a heme cofactor such as a heme-containing protein), to an uncooked food product, before and/or during the cooking process, to give the cooked consumable food product a particular meaty taste and smell, for example, the taste and smell of beef, bacon, pork, lamb, or chicken. Consumable food products can be animal or non-animal based (e.g., plant) food products, or combinations of an animal and non-animal based food product. For example, a plant based veggie burger or an animal-based burger, such as a chicken burger, can be modified with the compositions and methods of the present disclosure to result in a burger having a cooked flavor and/or aroma profile that is more meat like, e.g., beef-like, lamb-like, pork-like, turkey-like, duck-like, deer-like, yak-like, bison-like or other desirable meat flavor.

Food products for use in the present disclosure include those that have an iron-complex (e.g., a heme cofactor such as a heme-containing protein), and one or more flavor precursor molecules included therein. The iron-complex such as a heme cofactor (e.g., a heme-containing protein) and the one or more flavor precursor molecules can be homogeneously or heterogeneously included in the food products. A heme protein can be isolated and purified prior to inclusion in the food product. Non-limiting examples of consumable food products which can include an iron complex such as a heme-cofactor (e.g., a heme-containing protein) and one or more flavor precursor molecules include animal-based or non-animal (e.g., plant-based), or combinations of animal-based and non-animal-based, food products in the form of hot dogs, burgers, ground meat, sausages, steaks, filets, roasts, breasts, thighs, wings, meatballs, meatloaf, bacon, strips, fingers, nuggets, cutlets, or cubes.

Consumable food products for use in the present disclosure can be flavor additive compositions, e.g., for addition to another consumable food product before, during, or after its cooking process. A flavor additive composition can include an iron complex such as a heme-cofactor (e.g., a heme-containing protein), and one or more flavor precursors.

A flavor additive composition can include a heme protein, e.g., an isolated and purified heme protein; such a flavor additive composition can be used to modulate the flavor and/or aroma profile of a consumable food product that comprises one or more flavor precursor molecules or compositions. A flavor additive composition can include one or more flavor precursor molecules or compositions; such a flavor additive composition can be used to modulate the flavor and/or aroma profile of a consumable food product that comprises the heme protein, e.g., an isolated and purified heme protein.

A flavor additive composition can be in the form, of but not limited to, soup or stew bases, bouillon, e.g., powder or cubes, flavor packets, or seasoning packets or shakers. Such flavor additive compositions can be used to modulate the flavor and/or aroma profile for a variety of consumable food products, and can be added to a consumable food product before, during, or after cooking of the consumable food product.

In some embodiments, a flavor additive composition such as one including an iron complex (e.g., ferrous chlorin or a heme protein) and one or more flavor precursors can be reacted (e.g., in vitro) with heating to generate a particular flavor and/or aroma profile of interest and the resultant product mixture can be added to the consumable food product of interest, which can then be eaten as-is or can be additionally modified, e.g., by additional cooking. In some embodiments, the iron complex can be removed from the resultant product mixture before adding the product mixture to the consumable food product of interest. For example, the

iron complex can be removed from the product mixture using chromatographic techniques such as column chromatography, e.g., a column containing heme or iron-chlorin.

In some embodiments, the iron complex such as a heme-cofactor, e.g., a heme-protein, and the one or more flavor precursor flavor additive compositions can be soy-free, wheat-free, yeast-free, MSG-free, and free of protein hydrolysis products, and can taste meaty, highly savory, and without off odors or flavors.

In one aspect, this document features a food product that includes an iron complex such as a heme moiety, or a porphyrin, a porphyrinogen, a corrin, a corrinoid, a chlorin, a bacteriochlorophyll, a corphin, a chlorophyllin, a bacteriochlorin, or an isobacteriochlorin moiety complexed to an iron ion and one or more flavor precursor molecules selected from the group consisting of glucose, fructose, ribose, arabinose, glucose-6-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, inositol, maltose, sucrose, maltodextrin, glycogen, nucleotide-bound sugars, molasses, a phospholipid, a lecithin, inosine, inosine monophosphate (IMP), guanosine monophosphate (GMP), pyrazine, adenosine monophosphate (AMP), lactic acid, succinic acid, glycolic acid, thiamine, creatine, pyrophosphate, vegetable oil, algal oil, corn oil, soybean oil, palm fruit oil, palm kernel oil, safflower oil, flaxseed oil, rice bran oil, cottonseed oil, sunflower oil, canola oil, olive oil, a free fatty acid, cysteine, methionine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione, an amino acid derivative, a protein hydrolysate, a malt extract, a yeast extract, and a peptone. The heme moiety can be a heme-containing protein, a heme moiety bound to a non-peptidic polymer; or a heme moiety bound to a solid support. The heme-containing protein can be a plant, mammalian, a yeast or filamentous fungi, or bacterial heme-containing protein. The food product can include two to one hundred, two to fifty flavor precursors, two to forty flavor precursors, two to thirty-five flavor precursors, two to ten flavor precursors, or two to six flavor precursors. In some embodiments, the one or more flavor precursor molecules are selected from the group consisting of glucose, ribose, cysteine, a cysteine derivative, thiamine, alanine, methionine, lysine, a lysine derivative, glutamic acid, a glutamic acid derivative, IMP, GMP, lactic acid, maltodextrin, creatine, alanine, arginine, asparagine, aspartate, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, methionine, phenylalanine, proline, threonine, tryptophan, tyrosine, valine, linoleic acid, and mixtures thereof. The heme-containing protein can be a non-symbiotic hemoglobin or a leghemoglobin (e.g., a plant leghemoglobin such as one from soybean, alfalfa, lupin, pea, cow pea, or lupin). The heme-containing protein can include an amino acid sequence having at least 80% sequence identity to a polypeptide set forth in SEQ ID NOs:1-26. The heme-containing protein can be isolated and purified. The food product further can include a food-grade oil, a seasoning agent, a flavoring agent, a protein, a protein concentrate, an emulsifier, a gelling agent, or a fiber. The food product can be a meat substitute, a soup base, stew base, snack food, bouillon powder, bouillon cube, a flavor packet, or a frozen food product. Any of the food products can be free of animal products. The food product can be sealed within a packet or shaker.

This document also features a method for producing a flavor compound. The method can include combining an iron complex (e.g., a heme moiety, a porphyrin, a porphyrinogen, a corrin, a corrinoid, a chlorin, a bacteriochloro-

phyll, a corphin, a chlorophyllin, a bacteriochlorin, or an isobacteriochlorin complexed to an iron) and one or more flavor precursor molecules to form a mixture, the one or more flavor precursor molecules selected from the group consisting of glucose, fructose, arabinose, ribose glucose-6-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, inositol, maltose, sucrose, maltodextrin, glycogen, nucleotide-bound sugars, molasses, a phospholipid, a lecithin, inosine, inosine monophosphate (IMP), guanosine monophosphate (GMP), pyrazine, adenosine monophosphate (AMP), lactic acid, succinic acid, glycolic acid, thiamine, creatine, pyrophosphate, vegetable oil, algal oil, corn oil, soybean oil, palm fruit oil, palm kernel oil, safflower oil, flaxseed oil, rice bran oil, cottonseed oil, canola oil, olive oil, sunflower oil, flaxseed oil, coconut oil, mango oil, a free fatty acid, cysteine, methionine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione, an amino acid derivative, a protein hydrolysate, a malt extract, a yeast extract, and a peptone; and heating the mixture to form one or more flavor compounds selected from the group consisting of phenylacetaldehyde, 1-octen-3-one, 2-n-heptylfuran, 2-thiophenecarboxaldehyde, 3-thiophenecarboxaldehyde, butyrolactone, 2-undecenal, pyrazine, methyl-, furfural, 2-decanone, pyrrole, 1-octen-3-ol, 2-acetylthiazole, (E)-2-octenal, decanal, benzaldehyde, (E)-2-nonenal, pyrazine, 1-hexanol, 1-heptanol, dimethyl trisulfide, 2-nonanone, 2-pentanone, 2-heptanone, 2,3-butanedione, heptanal, nonanal, 2-octanone, 1-octanol, 3-ethylcyclopentanone, 3-octen-2-one, (E,E)-2,4-heptadienal, (Z)-2-heptenal, 2-heptanone, 6-methyl-, (Z)-4-heptenal, (E,Z)-2,6-nonadienal, 3-methyl-2-butanal, 2-pentyl-furan, thiazole, (E,E)-2,4-decadienal, hexanoic acid, 1-ethyl-5-methylcyclopentene, (E,E)-2,4-nonadienal, (Z)-2-decenal, dihydro-5-pentyl-2(3H)-furanone, trans-3-nonen-2-one, (E,E)-3,5-octadien-2-one, (Z)-2-octen-1-ol, 5-ethyldihydro-2(3H)-furanone, 2-butanal, 1-penten-3-ol, (E)-2-hexenal, formic acid, heptyl ester, 2-pentyl-thiophene, (Z)-2-nonenal, 2-hexyl-thiophene, (E)-2-decenal, 2-ethyl-5-methyl-pyrazine, 3-ethyl-2,5-dimethyl-pyrazine, 2-ethyl-1-hexanol, thiophene, 2-methyl-furan, pyridine, butanal, 2-ethyl-furan, 3-methyl-butanal, trichloromethane, 2-methyl-butanal, methacrolein, 2-methyl-propanal, propanal, acetaldehyde, 2-propyl-furan, dihydro-5-propyl-2(3H)-furanone, 1,3-hexadiene, 4-decyne, pentanal, 1-propanol, heptanoic acid, trimethyl-ethanethiol, 1-butan-1-ol, 1-penten-3-one, dimethyl sulfide, 2-ethyl furan, 2-pentyl-thiophene, 2-propenal, 2-tridecen-1-ol, 4-octene, 2-methyl thiazole, methyl-pyrazine, 2-butanone, 2-pentyl-furan, 2-methyl-propanal, butyrolactone, 3-methyl-butanal, methyl-thiirane, 2-hexyl-furan, butanal, 2-methyl-butanal, 2-methyl-furan, furan, octanal, 2-heptenal, 1-octene, formic acid heptyl ester, 3-pentyl-furan, and 4-penten-2-one. The heme moiety can be a heme-containing protein, a heme moiety bound to a non-peptidic polymer; or a heme moiety bound to a solid support. The method can include combining cysteine, ribose, lactic acid, lysine, and/or thiamine with the heme-containing protein.

In another aspect, this document features a method for producing a flavor compound. The method includes combining an iron complex, such as a heme-containing protein, and one or more flavor precursor molecules to form a mixture, the one or more flavor precursor molecules selected from the group consisting of glucose, fructose, ribose, arabinose, glucose-6-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, inositol, maltose, sucrose, maltodextrin, glycogen, nucleotide-bound sugars, molasses, a phos-

pholipid, a lecithin, inosine, IMP, GMP, pyrazine, AMP, lactic acid, succinic acid, glycolic acid, thiamine, creatine, pyrophosphate, vegetable oil, algal oil, corn oil, soybean oil, palm fruit oil, palm kernel oil, safflower oil, flaxseed oil, rice bran oil, cottonseed oil, olive oil, sunflower oil, canola oil, flaxseed oil, coconut oil, mango oil, a free fatty acid, methionine, cysteine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione, an amino acid derivative, a protein hydrolysate, a malt extract, a yeast extract, and a peptone; and heating the mixture to form one or more flavor compounds set forth in Tables 3, 8, or 9. For example, the flavor precursors can include cysteine, a sugar, and one or more other precursors.

This document also features a method for imparting a meat like flavor (e.g., beef-like, chicken like, pork-like, lamb-like, turkey-like, duck-like, deer-like, or bison-like) to a food product. The method includes contacting the food product with a flavoring composition, the flavoring composition comprising i) an iron complex, such as a heme moiety (e.g., a heme-containing protein); and ii) one or more flavor precursor molecules selected from the group consisting of glucose, fructose, ribose, arabinose, glucose-6-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, inositol, maltose, sucrose, maltodextrin, glycogen, nucleotide-bound sugars, molasses, a phospholipid, a lecithin, inosine, IMP, GMP, pyrazine, AMP, lactic acid, succinic acid, glycolic acid, thiamine, creatine, pyrophosphate, vegetable oil, algal oil, corn oil, soybean oil, palm fruit oil, palm kernel oil, safflower oil, flaxseed oil, rice bran oil, cottonseed oil, olive oil, sunflower oil, canola oil, flaxseed oil, coconut oil, mango oil, a free fatty acid, cysteine, methionine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione, an amino acid derivative, a protein hydrolysate, a malt extract, a yeast extract, and a peptone; wherein after heating the food product and the flavoring composition together, a meat like flavor (e.g., beef-like, chicken like, pork-like, lamb-like, turkey-like, duck-like, deer-like, or bison-like) is imparted to the food product. In some embodiments, the iron complex is removed from the food product. The flavoring composition further can include a seasoning agent, a flavoring agent, a protein, a protein concentrate, or an emulsifier. The flavoring composition can be sealed within a packet or shaker.

In another aspect, this document features a method of making a food product. The method includes combining an isolated heme-containing protein and one or more flavor precursor molecules to form a mixture, the one or more flavor precursor molecules selected from the group consisting of glucose, fructose, ribose, arabinose, glucose-6-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, inositol, maltose, sucrose, maltodextrin, glycogen, nucleotide-bound sugars, molasses, a phospholipid, a lecithin, inosine, IMP, GMP, pyrazine, AMP, lactic acid, succinic acid, glycolic acid, thiamine, creatine, pyrophosphate, sunflower oil, coconut oil, canola oil, flaxseed oil, mango oil, a free fatty acid, cysteine, methionine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione, an amino acid derivative, a protein hydrolysate, a malt extract, a yeast extract, and a peptone; and heating the mixture.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this

invention pertains. Although methods and materials similar or equivalent to those described herein can be used to practice the invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims. The word “comprising” in the claims may be replaced by “consisting essentially of” or with “consisting of,” according to standard practice in patent law.

DESCRIPTION OF THE DRAWINGS

FIG. 1 contains amino acid sequences of exemplary heme-containing proteins.

FIG. 2 is a bar graph of the beefiness rating of the meat replica with or without the Magic Mix, both samples in triplicate with 1% w/v LegH protein. Tasters rated beefiness on a scale from 1-7, with 1 being not beefy at all and 7 being exactly like ground beef.

DETAILED DESCRIPTION

This document is based on methods and materials for modulating the taste and/or aroma profile of food products. As described herein, compositions containing one or more flavor precursors and one or more highly conjugated heterocyclic rings complexed to an iron (referred to herein as an iron complex) can be used to modulate the taste and/or aroma profile of food products. Such iron complexes include heme moieties or other highly conjugated heterocyclic rings complexed to an iron ion (referred to as an iron complex). “Heme” refers to a prosthetic group bound to iron (Fe^{2+} or Fe^{3+}) in the center of a porphyrin ring. Thus, an iron complex can be a heme moiety, or a porphyrin, porphyrinogen, corrin, corrinoid, chlorin, bacteriochlorophyll, corphin, chlorophyllin, bacteriochlorin, or isobacteriochlorin moiety complexed to iron ion. The heme moiety that can be used to modulate the taste and/or aroma profile of food products can be a heme cofactor such as a heme-containing protein; a heme moiety bound to a non-peptidic polymer or other macromolecule such as a liposome, a polyethylene glycol, a carbohydrate, a polysaccharide, a cyclodextrin, a polyethylenimine, a polyacrylate, or derivatives thereof; a siderophore (i.e., an iron chelating compound); or a heme moiety bound to a solid support (e.g., beads) composed of a chromatography resin, cellulose, graphite, charcoal, or diatomaceous earth.

In some embodiments, the iron complexes catalyze some reactions and produce flavor precursors without heating or cooking. In some embodiments, the iron complex destabilizes upon heating or cooking and releases the iron, e.g., the protein is denatured, so flavor precursors can be generated.

Suitable flavor precursors include sugars, sugar alcohols, sugar derivatives, oils (e.g., vegetable oils), free fatty acids, alpha-hydroxy acids, dicarboxylic acids, amino acids and derivatives thereof, nucleosides, nucleotides, vitamins, peptides, protein hydrolysates, extracts, phospholipids, lecithin, and organic molecules. Non-limiting examples of such flavor precursors are provided in Table 1.

TABLE 1

Flavor Precursor Molecules	
5	Sugars, sugar alcohols, sugar acids, and sugar derivatives: glucose, fructose, ribose, sucrose, arabinose, glucose-6-phosphate, fructose-6-phosphate, fructose 1,6-diphosphate, inositol, maltose, molasses, maltodextrin, glycogen, galactose, lactose, ribitol, gluconic acid and glucuronic acid, amylose, amylopectin, or xylose
10	Oils: coconut oil, mango oil, sunflower oil, cottonseed oil, safflower oil, rice bran oil, cocoa butter, palm fruit oil, palm oil, soybean oil, canola oil, corn oil, sesame oil, walnut oil, flaxseed, jojoba oil, castor, grapeseed oil, peanut oil, olive oil, algal oil, oil from bacteria or fungi
15	Free fatty acids: caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid, palmitoleic acid, stearic, oleic acid, linoleic acid, alpha linolenic acid, gamma linolenic acid, arachidic acid, arachidonic acid, behenic acid, or erucic acid
	Amino acids and derivatives thereof: cysteine, cystine, a cysteine sulfoxide,
20	allicin, selenocysteine, methionine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, 5-hydroxytryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, or tyrosine
25	Nucleosides and Nucleotides: inosine, inosine monophosphate (IMP), guanosine, guanoside monophosphate (GMP), adenosine, adenosine monophosphate (AMP)
	Vitamins: thiamine, vitamin C, Vitamin D, Vitamin B6, or Vitamin E
	Misc: phospholipid, lecithin, pyrazine, creatine, pyrophosphate
	Acids: acetic acid, alpha hydroxy acids such as lactic acid or glycolic acid,
30	tricarboxylic acids such as citric acid, dicarboxylic acids such as succinic acid or tartaric acid
	Peptides and protein hydrolysates: glutathione, vegetable protein hydrolysates, soy protein hydrolysates, yeast protein hydrolysates, algal protein hydrolysates, meat protein hydrolysates
35	Extracts: a malt extract, a yeast extract, and a peptone

In some embodiments, one flavor precursor or combinations of two to one hundred flavor precursors, two to ninety, two to eighty, two to seventy, two to sixty, or two to fifty flavor precursors are used. For example, combinations of two to forty flavor precursors, two to thirty-five flavor precursors, two to ten flavor precursors, or two to six flavor precursors can be used with the one or more iron complexes (e.g., heme co-factors such as a heme-containing proteins). For example, the one or more flavor precursors can be glucose, ribose, cysteine, a cysteine derivative, thiamine, lysine, a lysine derivative, glutamic acid, a glutamic acid derivative, alanine, methionine, IMP, GMP, lactic acid, and mixtures thereof (e.g., glucose and cysteine; cysteine and ribose; cysteine, glucose or ribose, and thiamine; cysteine, glucose or ribose, IMP, and GMP; cysteine, glucose or ribose, and lactic acid). For example, the one or more flavor precursors can be alanine, arginine, asparagine, aspartate, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, threonine, tryptophan, tyrosine, valine, glucose, ribose, maltodextrin, thiamine, IMP, GMP, lactic acid, and creatine.

As used herein, the term “heme containing protein” can be used interchangeably with “heme containing polypeptide” or “heme protein” or “heme polypeptide” and includes any polypeptide that can covalently or noncovalently bind a heme moiety. In some embodiments, the heme-containing polypeptide is a globin and can include a globin fold, which comprises a series of seven to nine alpha helices. Globin type proteins can be of any class (e.g., class I, class II, or class III), and in some embodiments, can transport or store

oxygen. For example, a heme-containing protein can be a non-symbiotic type of hemoglobin or a leghemoglobin. A heme-containing polypeptide can be a monomer, i.e., a single polypeptide chain, or can be a dimer, a trimer, tetramer, and/or higher order oligomers. The life-time of the oxygenated Fe²⁺ state of a heme-containing protein can be similar to that of myoglobin or can exceed it by 10%, 20%, 30% 50%, 100% or more under conditions in which the heme-protein-containing consumable is manufactured, stored, handled or prepared for consumption. The life-time of the unoxygenated Fe²⁺ state of a heme-containing protein can be similar to that of myoglobin or can exceed it by 10%, 20%, 30% 50%, 100% or more under conditions in which the heme-protein-containing consumable is manufactured, stored, handled or prepared for consumption.

Non-limiting examples of heme-containing polypeptides can include an androglobin, a cytoglobin, a globin E, a globin X, a globin Y, a hemoglobin, a myoglobin, an erythrocyruorin, a beta hemoglobin, an alpha hemoglobin, a protoglobin, a cyanoglobin, a cytoglobin, a histoglobin, a neuroglobins, a chlorocruorin, a truncated hemoglobin (e.g., HbN or HbO), a truncated 2/2 globin, a hemoglobin 3 (e.g., Glb3), a cytochrome, or a peroxidase.

Heme-containing proteins that can be used in the compositions and food products described herein can be from mammals (e.g., farms animals such as cows, goats, sheep, pigs, ox, or rabbits), birds, plants, algae, fungi (e.g., yeast or filamentous fungi), ciliates, or bacteria. For example, a heme-containing protein can be from a mammal such as a farm animal (e.g., a cow, goat, sheep, pig, ox, or rabbit) or a bird such as a turkey or chicken. Heme-containing proteins can be from a plant such as *Nicotiana tabacum* or *Nicotiana sylvestris* (tobacco); *Zea mays* (corn), *Arabidopsis thaliana*, a legume such as *Glycine max* (soybean), *Cicer arietinum* (garbanzo or chick pea), *Pisum sativum* (pea) varieties such as garden peas or sugar snap peas, *Phaseolus vulgaris* varieties of common beans such as green beans, black beans, navy beans, northern beans, or pinto beans, *Vigna unguiculata* varieties (cow peas), *Vigna radiata* (Mung beans), *Lupinus albus* (lupin), or *Medicago sativa* (alfalfa); *Brassica napus* (canola); *Triticum* sps. (wheat, including wheat berries, and spelt); *Gossypium hirsutum* (cotton); *Oryza sativa* (rice); *Zizania* sps. (wild rice); *Helianthus annuus* (sunflower); *Beta vulgaris* (sugarbeet); *Pennisetum glaucum* (pearl millet); *Chenopodium* sp. (quinoa); *Sesamum* sp. (sesame); *Linum usitatissimum* (flax); or *Hordeum vulgare* (barley). Heme-containing proteins can be isolated from fungi such as *Saccharomyces cerevisiae*, *Pichia pastoris*, *Magnaporthe oryzae*, *Fusarium graminearum*, *Aspergillus oryzae*, *Trichoderma reesei*, *Myceliophthora thermophila*, *Kluyvera lactis*, or *Fusarium oxysporum*. Heme-containing proteins can be isolated from bacteria such as *Escherichia coli*, *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus megaterium*, *Synechocystis* sp., *Aquifex aeolicus*, *Methylobacterium infernorum*, or thermophilic bacteria such as *Thermophilus*. The sequences and structure of numerous heme-containing proteins are known. See for example, Reedy, et al., *Nucleic Acids Research*, 2008, Vol. 36, Database issue D307-D313 and the Heme Protein Database Available on the world wide web at hemeprotein.info/heme.php.

For example, a non-symbiotic hemoglobin can be from a plant selected from the group consisting of soybean, sprouted soybean, alfalfa, golden flax, black bean, black eyed pea, northern, garbanzo, moong bean, cowpeas, pinto beans, pod peas, quinoa, sesame, sunflower, wheat berries, spelt, barley, wild rice, or rice.

Any of the heme-containing proteins described herein that can be used for producing food products can have at least 70% (e.g., at least 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100%) sequence identity to the amino acid sequence of the corresponding wild-type heme-containing protein or fragments thereof that contain a heme-binding motif. For example, a heme-containing protein can have at least 70% sequence identity to an amino acid sequence set forth in FIG. 1, including a non-symbiotic hemoglobin such as that from *Vigna radiata* (SEQ ID NO:1), *Hordeum vulgare* (SEQ ID NO:5), *Zea mays* (SEQ ID NO:13), *Oryza sativa* subsp. *japonica* (rice) (SEQ ID NO:14), or *Arabidopsis thaliana* (SEQ ID NO:15), a Hell's gate globin I such as that from *Methylobacterium infernorum* (SEQ ID NO:2), a flavohemoprotein such as that from *Aquifex aeolicus* (SEQ ID NO:3), a leghemoglobin such as that from *Glycine max* (SEQ ID NO:4), *Pisum sativum* (SEQ ID NO:16), or *Vigna unguiculata* (SEQ ID NO:17), a heme-dependent peroxidase such as from *Magnaporthe oryzae*, (SEQ ID NO:6) or *Fusarium oxysporum* (SEQ ID NO:7), a cytochrome c peroxidase from *Fusarium graminearum* (SEQ ID NO:8), a truncated hemoglobin from *Chlamydomonas moewusii* (SEQ ID NO:9), *Tetrahymena pyriformis* (SEQ ID NO:10, group I truncated), *Paramecium caudatum* (SEQ ID NO:11, group I truncated), a hemoglobin from *Aspergillus niger* (SEQ ID NO:12), or a mammalian myoglobin protein such as the *Bos taurus* (SEQ ID NO:18) myoglobin, *Sus scrofa* (SEQ ID NO:19) myoglobin, *Equus caballus* (SEQ ID NO:20) myoglobin, a heme-protein from *Nicotiana benthamiana* (SEQ ID NO:21), *Bacillus subtilis* (SEQ ID NO:22), *Corynebacterium glutamicum* (SEQ ID NO:23), *Synechocystis* PCC6803 (SEQ ID NO:24), *Synechococcus* sp. PCC 7335 (SEQ ID NO:25), or *Nostoc commune* (SEQ ID NO:26).

The percent identity between two amino acid sequences can be determined as follows. First, the amino acid sequences are aligned using the BLAST 2 Sequences (B12seq) program from the stand-alone version of BLASTZ containing BLASTP version 2.0.14. This stand-alone version of BLASTZ can be obtained from Fish & Richardson's web site (e.g., www.fr.com/blast/) or the U.S. government's National Center for Biotechnology Information web site (www.ncbi.nlm.nih.gov). Instructions explaining how to use the B12seq program can be found in the readme file accompanying BLASTZ. B12seq performs a comparison between two amino acid sequences using the BLASTP algorithm. To compare two amino acid sequences, the options of B12seq are set as follows: -i is set to a file containing the first amino acid sequence to be compared (e.g., C:\seq1.txt); -j is set to a file containing the second amino acid sequence to be compared (e.g., C:\seq2.txt); -p is set to blastp; -o is set to any desired file name (e.g., C:\output.txt); and all other options are left at their default setting. For example, the following command can be used to generate an output file containing a comparison between two amino acid sequences: C:\B12seq -i c:\seq1.txt -j c:\seq2.txt -p blastp -o c:\output.txt. If the two compared sequences share homology, then the designated output file will present those regions of homology as aligned sequences. If the two compared sequences do not share homology, then the designated output file will not present aligned sequences. Similar procedures can be following for nucleic acid sequences except that blastn is used.

Once aligned, the number of matches is determined by counting the number of positions where an identical amino acid residue is presented in both sequences. The percent identity is determined by dividing the number of matches by

11

the length of the full-length polypeptide amino acid sequence followed by multiplying the resulting value by 100. It is noted that the percent identity value is rounded to the nearest tenth. For example, 78.11, 78.12, 78.13, and 78.14 is rounded down to 78.1, while 78.15, 78.16, 78.17, 78.18, and 78.19 is rounded up to 78.2. It also is noted that the length value will always be an integer.

It will be appreciated that a number of nucleic acids can encode a polypeptide having a particular amino acid sequence. The degeneracy of the genetic code is well known to the art; i.e., for many amino acids, there is more than one nucleotide triplet that serves as the codon for the amino acid. For example, codons in the coding sequence for a given enzyme can be modified such that optimal expression in a particular species (e.g., bacteria or fungus) is obtained, using appropriate codon bias tables for that species.

Heme-containing proteins can be extracted from the source material (e.g., extracted from animal tissue, or plant, fungal, algal, or bacterial biomass, or from the culture supernatant for secreted proteins) or from a combination of source materials (e.g., multiple plant species). Leghemoglobin is readily available as an unused by-product of commodity legume crops (e.g., soybean, alfalfa, or pea). The amount of leghemoglobin in the roots of these crops in the United States exceeds the myoglobin content of all the red meat consumed in the United States.

In some embodiments, extracts of heme-containing proteins include one or more non-heme-containing proteins from the source material (e.g., other animal, plant, fungal, algal, or bacterial proteins) or from a combination of source materials (e.g., different animal, plant, fungi, algae, or bacteria).

In some embodiments, heme-containing proteins are isolated and purified from other components of the source material (e.g., other animal, plant, fungal, algal, or bacterial proteins). As used herein, the term "isolated and purified" indicates that the preparation of heme-containing protein is at least 60% pure, e.g., greater than 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% pure. Without being bound by theory, isolating and purifying proteins can allow the food products to be made with greater consistency and greater control over the properties of the food product as unwanted material is eliminated. Proteins can be separated on the basis of their molecular weight, for example, by size exclusion chromatography, ultrafiltration through membranes, or density centrifugation. In some embodiments, the proteins can be separated based on their surface charge, for example, by isoelectric precipitation, anion exchange chromatography, or cation exchange chromatography. Proteins also can be separated on the basis of their solubility, for example, by ammonium sulfate precipitation, isoelectric precipitation, surfactants, detergents or solvent extraction. Proteins also can be separated by their affinity to another molecule, using, for example, hydrophobic interaction chromatography, reactive dyes, or hydroxyapatite. Affinity chromatography also can include using antibodies having specific binding affinity for the heme-containing protein, nickel NTA for His-tagged recombinant proteins, lectins to bind to sugar moieties on a glycoprotein, or other molecules which specifically binds the protein.

Heme-containing proteins also can be recombinantly produced using polypeptide expression techniques (e.g., heterologous expression techniques using bacterial cells, insect cells, fungal cells such as yeast, plant cells such as tobacco, soybean, or *Arabidopsis*, or mammalian cells). In some cases, standard polypeptide synthesis techniques (e.g., liquid-phase polypeptide synthesis techniques or solid-phase

12

polypeptide synthesis techniques) can be used to produce heme-containing proteins synthetically. In some cases, in vitro transcription-translation techniques can be used to produce heme-containing proteins.

The protein used in the consumable may be soluble in a solution. In some embodiments, the isolated and purified proteins are soluble in solution at greater than 5, 10, 15, 20, 25, 50, 100, 150, 200, or 250 g/L.

In some embodiments, the isolated and purified protein is substantially in its native fold and water soluble. In some embodiments, the isolated and purified protein is more than 50, 60, 70, 80, or 90% in its native fold. In some embodiments, the isolated and purified protein is more than 50, 60, 70, 80, or 90% water soluble.

In some embodiments, the food product contains between 0.01% and 5% by weight of a heme protein. In some embodiments, the food product contains between 0.01% and 5% by weight of leghemoglobin. Some meat also contains myoglobin, a heme protein, which accounts for most of the red color and iron content of some meat. It is understood that these percentages can vary in meat and the food products can be produced to approximate the natural variation in meat.

In some embodiments, the food product comprises about 0.05%, about 0.1%, about 0.2%, about 0.3%, about 0.4%, about 0.5%, about 0.6%, about 0.7%, about 0.8%, about 0.9%, about 1%, about 1.1%, about 1.2%, about 1.3%, about 1.4%, about 1.5%, about 1.6%, about 1.7%, about 1.8%, about 1.9%, about 2%, or more than about 2% of an iron-carrying protein (e.g., a heme-containing protein) by dry weight or total weight. In some cases, the iron carrying protein has been isolated and purified from a source.

Modulating Flavor and/or Aroma Profiles

As described herein, different combinations of flavor precursors can be used with one or more iron complexes (e.g., a ferrous chlorin, a chlorin-iron complex, or a heme-cofactor such as a heme-containing protein or heme bound to a non-peptidic polymer such as polyethylene glycol or to a solid support) to produce different flavor and aroma profiles when the flavor precursors and iron complexes are heated together (e.g., during cooking). The resultant flavor and/or aroma profile can be modulated by the type and concentration of the flavor precursors, the pH of the reaction, the length of cooking, the type and amount of iron complex (e.g., a heme-cofactor such as heme-containing protein, heme bound to non-peptidic polymer or macromolecule, or heme bound to a solid support), the temperature of the reaction, and the amount of water activity in the product, among other factors. In embodiments in which a heme moiety is bound to a solid support such as cellulose or a chromatography resin, graphite, charcoal, or diatomaceous earth, the solid support (e.g., beads) can be incubated with sugars and/or one or more other flavor precursors to generate flavors, and then the solid support with attached heme moiety can be re-used, i.e., incubated again with sugars and/or one or more other flavor precursors to generate flavors.

Table 2 provides non-limiting examples of flavor types that can be generated by combining one or more flavor precursors and one or more heme co-factors (e.g., heme-containing proteins). See also Tables 7 and/or 11.

TABLE 2

Flavor Types

beef	beef broth
beef dripping	cheesy

13

TABLE 2-continued

Flavor Types	
cold-cut deli meat	squash
bacon	sharp
meaty	fruity
brothy	floral
ramen	musty
egg	fried food
malty	caramel
bready	barbeque
sulfur	chocolate
fried chicken	sweet
browned	potato
pretzel	french toast
grassy	breadcrust
bloody	mushroom
broccoli	chicken
brothy	cumin
buttery	umami
metallic	raisin
yeasty	goaty
vegetable broth	

Flavor and aroma profiles are created by different chemical compounds formed by chemical reactions between the heme co-factor (e.g., heme-containing protein) and flavor precursors. Gas chromatography—mass spectrometry (GCMS) can be used to separate and identify the different chemical compounds within a test sample. For example, volatile chemicals can be isolated from the head space after heating a heme-containing protein and one or more flavor precursors.

Table 3 provides non-limiting examples of compounds that can be produced. See also Tables 8, 9, 12, and/or 14.

TABLE 3

Compounds Produced		
phenylacetaldehyde	2-butenal, 2-ethyl-	1,3-hexadiene
1-octen-3-one	acetone	4-decyne
2-n-heptylfuran	pentanal	pentanal
2-thiophenecarboxaldehyde	(E)-2-Hexenal	1-propanol
3-thiophenecarboxaldehyde	4-ethyl-phenol,	heptanoic acid
1-octene	3-octanone	ethanethiol
butyrolactone	styrene	2-methyl-1-heptene
2-undecenal	furan, 3-pentyl-	(E)-4-octene
propyl-cyclopropane	fornic acid, heptyl ester	2-methyl-2-heptene
methyl-pyrazine	(E)-2-Heptenal	pentanoic acid
1-hydroxy-propanone	6-methyl-5-hepten-2-one	nonanoic acid
acetic acid	n-caproic acid vinyl ester	1,3-dimethyl-benzene
furfural	2-ethyl-2-hexenal	
2-decanone	1-hepten-3-ol	toluene
pyrrole	1-ethyl-1-methyl-cyclopentane	1-butanol
1-octen-3-ol	3-ethyl-2-methyl-1,3-hexadiene	2,3,3-trimethyl-pentane
2-acetylthiazole	2-pentyl-thiophene	isopropyl alcohol
(E)-2-octenal	(Z)-2-nonenal	2,2,4,6,6-pentamethyl-heptane
decanal	2-n-octylfuran	phenol
benzaldehyde	2-hexyl-thiophene	1-penten-3-one
(E)-2-Nonenal	4-cyclopentene-1,3-dione	dimethyl sulfide
pyrazine	1-nonanol	thiirane
1-pentanol	(E)-2-decenal	(E)-2-octen-1-ol
trans-2-(2-pentenyl)furan	4-ethyl-benzaldehyde	2,4-dimethyl-1-heptene
1-hexanol	1,7-octadien-3-ol	1,3-bis(1,1-dimethylethyl)-benzene
1-heptanol	octanoic acid	heptane
dimethyl trisulfide	2-ethyl-5-methyl-pyrazine	4,7-dimethyl-undecane

14

TABLE 3-continued

Compounds Produced		
2-nonanone	3-ethyl-2,5-dimethyl-pyrazine	acetophenone
2-pentanone	1,3,5-cycloheptatriene	tridecane
2-heptanone	2-ethyl-1-hexanol	thiophosphoramidate, s-methyl ester
2,3-butanedione	4-methyl-octanoic acid	2-methyl-thiazole
heptanal	m-aminophenylacetylene	3-(1-methylethoxy)-propanenitrile,
10 nonanal	benzene	2,4-bis(1,1-dimethylethyl)-phenol
2-octanone	thiophene	3-ethyl-2,2-dimethyl-pentane
2-butanone	2-methyl-furan	3-ethyl-pentane
15 octanal	pyridine	2,3,4-trimethyl-pentane
1-octanol	furan	2,4,6-trimethyl-octane
3-ethylcyclopentanone	butanal	2,6-dimethyl-nonane
8-methyl-1-undecene	2-ethyl-furan	2-hexyl-furan
3-octen-2-one	carbon disulfide	4-methyl-5-thiazoleethanol
20 2,4-Heptadienal, (E,E)-(Z)-2-heptenal	Furan, 2-hexyl-:2	4-penten-2-one
6-methyl-2-heptanone	3-methyl-butanol	3-methylthiazole
	2-methyl-butanol	2-methyl-3-pentanone
(Z)-4-heptenal	methacrolein	2,3-pentanedione
25 (E,E)-2,6-nonadienal	octane	(E)-2-tridecen-1-ol
3-methyl-2-butanol	ethanol	2-thio-phenemethanamine (Z)-2-nonenal,
2-pentyl-furan	2-methyl-propanal	methyl thiolacetate
thiazole	acetone	methyl ethanoate
(E,E)-2,4-decadienal	propanal	isothiazole
30 hexanoic acid	methyl-thiirane	3,3-dimethyl-hexane
1-ethyl-5-methylcyclopentene	acetaldehyde	
(E,E)-2,4-nonadienal	2-propenal	4-methyl-heptane
(Z)-2-decenal	2-propyl-furan	2,4-dimethyl-heptane
35 dihydro-5-pentyl-2(3h)-furanone	dihydro-5-propyl-2(3H)-furanone	2,3,4-trimethyl-heptane
trans-3-nonen-2-one	dihydro-3-(2H)-thiophenone	2-methyl-heptane
(E,E)-3,5-octadien-2-one	2,2,6-trimethyl-decane	2-methyl-3-furanthiol
(Z)-2-octen-1-ol	3,3'-dithiobis[2-methyl-furan	4-amino-1,2,5-oxadiazole-3-carbonitrile
40 5-ethyl-dihydro-2(3h)-furanone	1-heptene	1,2-benzisothiazol-3(2H)-one
2-butenal	1,3-octadiene	2-acetyl-propen-2-ol,
1-penten-3-ol	1-nonene	1-decen-3-one
1-(ethylthio)-2-(methylthio)-buta-1,3-diene		

In some embodiments, an iron complex (e.g., a ferrous chlorin or a heme-cofactor such as a heme-containing protein) described herein is heated in the presence of ground chicken, to increase specific volatile flavor and odorant components typically elevated in beef. For example, propanal, butanal, 2-ethyl-furan, heptanal, octanal, trans-2-(2-pentenyl)furan, (Z)-2-heptenal, (E)-2-octenal, pyrrole, 2,4-dodecadienal, 1-octanal, (Z)-2-decenal, or 2-undecenal can be increased in the presence of the heme-containing protein, which can impart a more beefy flavor to the chicken.

In some embodiments, an iron complex (e.g., a ferrous chlorin or a heme-cofactor such as a heme-containing protein) described herein is heated in the presence of cysteine and glucose or other combinations of flavor precursors to provide a different profile of volatile odorants than when any subset of the three components are used individually. Volatile flavor components that are increased under these conditions include but are not limited to furan, acetone, thiazole, furfural, benzaldehyde, 2-pyridinecarboxaldehyde, 5-methyl-2-thiophenecarboxaldehyde, 3-methyl-2-thio-

15

phenecarboxaldehyde, 3-thiophenemethanol and decanol. See, e.g., Tables 8 and 9. Under these conditions, cysteine and glucose alone or in the presence of iron salts such as ferrous glucanate produced a sulfurous, odor, but addition of heme-containing proteins reduced the sulfurous odor and replaced it with flavors including but not limited to chicken

In some embodiments, an iron complex (e.g., a ferrous chlorin or a heme-cofactor such as a heme-containing protein) described herein is heated in the presence of cysteine and ribose to provide a different profile of volatile odorants. Heating in the presence of ribose created some additional compounds as compared to when a heme-containing protein and glucose were heated together. See Tables 8 and 9.

In some embodiments, an iron complex (e.g., a ferrous chlorophyllin or a heme-cofactor such as a heme-containing protein) described herein can be heated in the presence of thiamine and a sugar to affect the formation of 5-Thiazoleethanol, 4-methyl-furan, 3,3'-dithiobis[2-methyl-furan, and/or 4-Methylthiazole. These compounds are known to be present in meat and have beefy, meaty taste notes.

In some embodiments, an iron complex (e.g., a ferrous chlorin or a heme-cofactor such as a heme-containing protein) described herein can be heated in the presence of a nucleotide such as inosine monophosphate and/or guanosine monophosphate to control the formation of flavor compounds such as (E)-4-octene, 2-ethyl-furan, 2-pentanone, 2,3-butanedione, 2-methyl-thiazole, methyl-pyrazine, tridecane, (E)-2-octenal, 2-thiopenecarboxaldehyde, and/or 3-thiopenecarboxaldehyde. These compounds are known to be present in meat and have a beefy, meaty, buttery, and or savory flavor notes.

In some embodiments, an iron complex (e.g., a ferrous chlorin or a heme-cofactor such as a heme-containing protein) described herein can be heated in the presence of lysine, a sugar such as ribose, and cysteine to control the formation of flavor compounds such as dimethyl trisulfide, nonanal, 2-pentyl thiophene, 2-nonenal furfural, 1-octanol, 2-nonenal, thiazole, 2-acetylthiazole, phenylacetaldehyde, and/or 2-acetylthiazole. These compounds are known to be present in meat and some have a beefy, meaty, and or savory flavor.

In some embodiments, an iron complex (e.g., a ferrous chlorin or a heme-cofactor such as a heme-containing protein) described herein can be heated in the presence of lactic acid, a sugar such as ribose, and cysteine to control the formation of the flavor compounds nonanal, thiazole, 2-acetylthiazole, and/or 8-methyl 1-undecene. These compounds are known to be present in meat and have beefy, savory, browned, bread, and malty notes.

In some embodiments, an iron complex (e.g., a ferrous chlorin or a heme-cofactor such as a heme-containing protein) described herein can be heated in the presence of amino acids, sugars such as glucose, ribose, and maltodextrin, lactic acid, thiamine, IMP, GMP, creatine, and salts such as potassium chloride and sodium chloride, to control the formation of flavor compounds such as 1,3-bis(1,1-dimethylethyl)-benzene, 2-methyl 3-furanthiol, and/or bis(2-methyl-4,5-dihydro-3-furyl)disulfide. These compounds are known to be present in meat and have beefy notes. See also Table 14.

In some embodiments, a particular type of heme-containing protein is chosen to control the formation of flavor compounds. See, for example, the results of Table 9, which shows that the addition of different types of heme-proteins (LegH, Barley, *B. myoglobin*, or *A. aeolicus*) in flavor reaction mixtures containing one or more flavor precursor

16

compounds results in many of the same key meat flavors, including but not limited to pentanone, 3-methyl butanal, 2-methyl butanal, 2-heptenal, 1-octene, nonanal, 2-propenal, 2-decenal, 2-nonanone, 2-octanone, 2-tridecen-1-ol, 2-octanone, 2-octenal, 4-methyl-2-heptanone, octanal, 2-undecenal, butyrolactone, 1-octen-3-one, 3-methylheptyl acetate, and 2-pentyl-thiophene. These differences in flavor compounds can change the overall taste profile.

In some embodiments, an iron complex (e.g., a ferrous chlorin or a heme-cofactor such as a heme-containing protein) described herein and one or more flavor precursors can be reacted (e.g., in vitro) with heating to generate a particular flavor and/or aroma profile of interest and the resultant flavor additive composition can be added to the consumable food product of interest, which can then be eaten as-is or can be additionally modified, e.g., by additional cooking.

In some embodiments, any undesirable flavors can be minimized by deodorizing with activated charcoal or by removing enzymes such as lipoxygenases (LOX), which can be present in trace amounts when using preparations of plant proteins, and which can convert unsaturated triacylglycerides (such as linoleic acid or linolenic acid) into smaller and more volatile molecules. LOX are naturally present in legumes such as peas, soybeans, and peanuts, as well as rice, potatoes, and olives. When legume flours are fractionated into separate protein fractions, LOX can act as undesirable "time-bombs" that can cause undesirable flavors on aging or storage. Compositions containing plant proteins (e.g., from ground plant seeds) can be subjected to purification to remove LOX using, for example, an affinity resin that binds to LOX and removes it from the protein sample. The affinity resin can be linoleic acid, linolenic acid, stearic acid, oleic acid, propyl gallate, or epigallocatechin gallate attached to a solid support such as a bead or resin. See, e.g., WO2013138793. In addition, depending on the protein component of the food product, certain combinations of antioxidants and/or LOX inhibitors can be used as effective agents to minimize off-flavor or off-odor generation especially in the presence of fats and oils. Such compounds can include, for example, one or more of β -carotene, α -tocopherol, caffeic acid, propyl gallate, or epigallocatechin gallate.

In some embodiments, specific flavor compounds, such as those described in Tables 3, 8, 9, 12, 14, 16, or 17 can be isolated and purified from the flavor additive composition. These isolated and purified compounds can be used as an ingredient to create flavors useful to the food and fragrance industry.

A flavor additive composition can be in the form, of but not limited to, soup or stew bases, bouillon, e.g., powder or cubes, flavor packets, or seasoning packets or shakers. Such flavor additive compositions can be used to modulate the flavor and/or aroma profile for a variety of food products, and can be added to a consumable food product before, during, or after cooking of the food product.

Food Products

Food products containing one or more flavor precursors and one or more heme-containing proteins can be used as a base for formulating a variety of additional food products, including meat substitutes, soup bases, stew bases, snack foods, bouillon powders, bouillon cubes, flavor packets, or frozen food products. Meat substitutes can be formulated, for example, as hot dogs, burgers, ground meat, sausages, steaks, filets, roasts, breasts, thighs, wings, meatballs, meatloaf, bacon, strips, fingers, nuggets, cutlets, or cubes.

In addition, food products described herein can be used to modulate the taste and/or aroma profile of other food products (e.g., meat replicas, meat substitutes, tofu, mock duck

or other gluten based vegetable product, textured vegetable protein such as textured soy protein, pork, fish, lamb, or poultry products such as chicken or turkey products) and can be applied to the other food product before or during cooking. Using the food products described herein can provide a particular meaty taste and smell, for example, the taste and smell of beef or bacon, to a non-meat product or to a poultry product.

Food products described herein can be packaged in various ways, including being sealed within individual packets or shakers, such that the composition can be sprinkled or spread on top of a food product before or during cooking.

Food products described herein can include additional ingredients including food-grade oils such as canola, corn, sunflower, soybean, olive or coconut oil, seasoning agents such as edible salts (e.g., sodium or potassium chloride) or herbs (e.g., rosemary, thyme, basil, sage, or mint), flavoring agents, proteins (e.g., soy protein isolate, wheat gluten, pea vicilin, and/or pea legumin), protein concentrates (e.g., soy protein concentrate), emulsifiers (e.g., lecithin), gelling agents (e.g., k-carrageenan or gelatin), fibers (e.g., bamboo fiber or inulin), or minerals (e.g., iodine, zinc, and/or calcium).

Food products described herein also can include a natural coloring agent such as turmeric or beet juice, or an artificial coloring agent such as azo dyes, triphenylmethanes, xanthenes, quinines, indigoids, titanium dioxide, red #3, red #40, blue #1, or yellow #5.

Food products described herein also can include meat shelf life extenders such as carbon monoxide, nitrites, sodium metabisulfite, Bombal, vitamin E, rosemary extract, green tea extract, catechins and other anti-oxidants.

Food products described herein can be free of animal products (e.g., animal heme-containing proteins or other animal products).

In some embodiments, the food products can be soy-free, wheat-free, yeast-free, MSG-free, and/or free of protein hydrolysis products, and can taste meaty, highly savory, and without off odors or flavors.

Assessment of Food Products

Food products described herein can be assessed using trained human panelists. The evaluations can involve eyeing, feeling, chewing, and tasting of the product to judge product appearance, color, integrity, texture, flavor, and mouth feel, etc. Panelists can be served samples under red or under white light. Samples can be assigned random three-digit numbers and rotated in ballot position to prevent bias. Sensory judgments can be scaled for "acceptance" or "likeability" or use special terminology. For example, letter scales (A for excellent, B for good, C for poor) or number scales may be used (1=dislike, 2=fair, 3=good; 4=very good; 5=excellent). A scale can be used to rate the overall acceptability or quality of the food product or specific quality attributes such as beefiness, texture, and flavor. Panelists can be encouraged to rinse their mouths with water between samples, and given opportunity to comment on each sample.

In some embodiments, a food product described herein can be compared to another food product (e.g., meat or meat substitute) based upon olfactometer readings. In various embodiments, the olfactometer can be used to assess odor concentration and odor thresholds, odor suprathresholds with comparison to a reference gas, hedonic scale scores to determine the degree of appreciation, or relative intensity of odors.

In some embodiments, an olfactometer allows the training and automatic evaluation of expert panels. In some embodiments, a food product described herein causes similar or

identical olfactometer readings. In some embodiments, the differences between flavors generated using the methods of the invention and meat are sufficiently small to be below the detection threshold of human perception.

In some embodiments, volatile chemicals identified using GCMS can be evaluated. For example, a human can rate the experience of smelling the chemical responsible for a certain peak. This information could be used to further refine the profile of flavor and aroma compounds produced using a heme-containing protein and one or more flavor precursors.

Characteristic flavor and fragrance components are mostly produced during the cooking process by chemical reactions molecules including amino acids, fats and sugars which are found in plants as well as meat. Therefore, in some embodiments, a food product is tested for similarity to meat during or after cooking. In some embodiments human ratings, human evaluation, olfactometer readings, or GCMS measurements, or combinations thereof, are used to create an olfactory map of the food product. Similarly, an olfactory map of the food product, for example, a meat replica, can be created. These maps can be compared to assess how similar the cooked food product is to meat.

In some embodiments, the olfactory map of the food product during or after cooking is similar to or indistinguishable from that of cooked or cooking meat. In some embodiments the similarity is sufficient to be beyond the detection threshold of human perception. The food product can be created so its characteristics are similar to a food product after cooking, but the uncooked food product may have properties that are different from the predicate food product prior to cooking.

These results will demonstrate that the compositions of the invention are judged as acceptably equivalent to real meat products. Additionally, these results can demonstrate that compositions of the invention are preferred by panelist over other commercially available meat substitutes. So, in some embodiments the present invention provides for consumables that are significantly similar to traditional meats and are more meat like than previously known meat alternatives.

The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims.

EXAMPLES

Example 1: Addition of Heme-Protein Increases Beefy Qualities of Replica Burgers

Replica burgers containing the ingredients in Table 4 and the flavor precursors cysteine (10 mM), glutamic acid (10 mM), glucose (10 mM), and thiamine (1 mM) were prepared. Water was added to make up the balance. See, for example, U.S. Provisional Application No. 61/751,816, filed Jan. 11, 2013. Control burgers were prepared as in Table 4 with precursors cysteine (10 mM), glutamic acid (10 mM), glucose (10 mM), and thiamine (1 mM) except LegH was omitted.

After cooking for 5 minutes at 150 C, the replica burgers were evaluated by a trained sensory panel. Panelists were served samples under red lights and each panelist individually evaluated the samples. Samples were assigned a random three-digit number and rotated in ballot position to prevent bias. Panelists were asked to evaluate cooked replica burger samples on multiple flavor, aroma, taste, texture and appearance attributes including but not limited to: beefiness, bloody quality, savory quality, and overall acceptability

19

using a 7-point scale from 1=dislike extremely, to 7=like extremely. Panelists were encouraged to rinse their mouths with water between samples, and to fill out a survey to record their evaluation of each sample.

When replica burgers containing the LegH were compared to the control replica burgers without LegH, the samples containing LegH were rated significantly beefier, bloodier, more savory, and overall preferred compared to those that did not include LegH. See Table 5.

TABLE 4

Replica Burger Ingredients	
Replica burger	% precooked w/w
Pea vicilin	3.86
Soy protein concentrate (SPC)	2.52
Bamboo fiber	0.34
NaCl	0.54
Pea legumin	2
Soy Protein Isolate (SPI) (Solae, St. Louis, MO)	4.68
Wheat gluten	4.68
Coconut oil	15
Soy lecithin	0.1
k-carrageenan	1
LegH	1

TABLE 5

Sensory evaluation of replica burger with Heme			
Attribute	Beef 20/80	No Heme	1% Heme
<u>Beefyness</u>			
mean	5.33	1.30	3.20
STDEV	1.58	0.67	0.79
<u>Bloody</u>			
mean	4.00	1.10	2.78
STDEV	1.32	0.32	1.64
<u>Savory</u>			
mean	4.67	3.00	5.10
STDEV	1.22	1.63	0.57

Example 2: Replica Burgers with a Flavor Precursor Mixture Taste Beefy and Bloody

Replica burgers containing a flavor precursor mixture of glucose, cysteine, thiamine, and glutamic acid and 1% LegH

20

pre-cooked w/w (see Table 4) were prepared as described in Example 1, and evaluated by a trained sensory panel after the burgers were cooked for 5 minutes at 150 C. Control burgers included LegH and all other ingredients except for the flavor precursor mixture.

Panelists were asked to evaluate the samples overall improvement in taste and descriptively analyze each sample using a 5-point scale from 1=dislike extremely, to 5=like extremely. Panelists were encouraged to rinse their mouths with water between samples, and to fill out a survey to record their evaluation of each sample. The replicate burgers which included LegH and the flavor precursor mixture were described as having bouillon, gravy, meaty, bloody, savory, and beefy notes on taste, and were preferred to the same replica burger with LegH but no added flavor precursor mixture. See, Table 6

TABLE 6

Improvement of overall taste with precursors added to LegH burgers		
	with precursors	without precursors
Average	3.5	1.8
STDV	0.6	0.5

Example 3: Replica Burgers with Flavor Precursor Mixture Resulting in a Bacon Taste

Replica burgers (see Table 4) were cooked with different precursor mixes (see Table 7) and 1% LegH and evaluated by a trained sensory panel after the burgers were cooked for 5 minutes at 150 C. Control burgers contained LegH and all of the other ingredients except for the flavor precursors. Panelists were asked to evaluate each sample and descriptively analyze of each sample. 5-point scale from 1=dislike extremely, to 5=like extremely. Panelists were encouraged to rinse their mouths with water between samples, and to fill out a survey to record their evaluation of each sample. A replica burger with a precursor mixture of 10 mM glucose, 10 mM ribose, 10 mM cysteine, 1 mM thiamine, 1 mM glutamic acid, 1 mM GMP, and LegH was described as having a bacon aroma and taste, and overall meatiness, savory quality, a very umami quality, a brothy quality, and slight beefy notes. See Table 7 for a summary of the flavor description for the various combinations of flavor precursors and heme-containing protein.

TABLE 7

Flavors generated by addition of precursors to LegH (1%)			
Precursor (concentration)		Flavor Description	
ribose (10 mM)	cysteine (10 mM)	some kind of cold-cut/sliced deli meat	
ribose (10 mM)	cysteine (10 mM)	IMP (2 mM)	bread crust with beef drippings, sweet, grassy, umami
ribose (10 mM)	cysteine (10 mM)	lactic acid (1 mM)	bready, malty, browned, breadcrust
ribose (10 mM)	cysteine (10 mM)	lysine (5 mM)	savory, beefy, little grassy, brothy, bread
ribose (10 mM)	cysteine (10 mM)	alanine (5 mM)	savory, weak beefy, brothy, little metallic
ribose (10 mM)	cysteine (10 mM)	I + G (2 mM)	savory, weak beefy, brothy, sweet

TABLE 7-continued

Flavors generated by addition of precursors to LegH (1%)						
Precursor (concentration)				Flavor Description		
	ribose (10 mM)	cysteine (10 mM)		methionine	cooked potato	
	ribose (10 mM)	cysteine (10 mM)	glutamic acid (5 mM)		little meaty, pretzel, brothy, savory, sweet, chocolate	
glucose (10 mM)	ribose (10 mM)	cysteine (10 mM)	thiamine (2 mM)	glutamic acid (5 mM)	slight beefy, browned, grassy,	
glucose (10 mM)	ribose (10 mM)	cysteine (10 mM)	thiamine (2 mM)	glutamic acid (5 mM)	IMP (2 mM)	bacon, very umami, savory, brothy, slight beef
glucose (10 mM)		cysteine (10 mM)	thiamine (2 mM)	glutamic acid (5 mM)		beef jerky, bloody, meaty, brothy
glucose (10 mM)		cysteine (10 mM)	thiamine (2 mM)	glutamic acid (5 mM)	lactic acid (1 mM)	savory, beefy, bloody, meaty, savory, gravy
glucose (10 mM)		cysteine (10 mM)	thiamine (2 mM)	glutamic acid (5 mM)	lysine (5 mM)	roast beef
glucose (10 mM)		cysteine (10 mM)	thiamine (2 mM)	glutamic acid (5 mM)	alanine (5 mM)	boiled beef, sweet
glucose (10 mM)		cysteine (10 mM)	thiamine (2 mM)	glutamic acid (5 mM)	I + G (2 mM)	beefy with a sulfury note
glucose (10 mM)		cysteine (10 mM)		I + G (2 mM)		sweet, malty, umami, meaty
glucose (10 mM)				I + G (2 mM)		savory, roast beef, grassy
glucose (10 mM)			glutamic acid (5 mM)			umami, savory, meaty, sweaty, fermented

Example 4: Type of Sugar Modulates Flavor
Compounds Created in the Presence of
Hemeprotein

The addition of different sugars to flavor reaction mixtures containing a hemeprotein and one or more flavor precursor compounds resulted in distinct differences in the flavor compounds generated and the overall flavor profile. LegH heme protein at 1% pre-cooked w/w/ was mixed with cysteine (10 mM) and glucose (20 mM) at pH 6 in phosphate buffer to form a flavor reaction mixture and heated to 150 C for 3 minutes; this reaction created flavor compounds known to be present in meat; see Table 8. Similarly, a flavor reaction mixture made when LegH heme protein at 1% was mixed with cysteine (10 mM) and ribose (20 mM) at pH 6 and heated to 150 C for 3 minutes created flavor compounds known to be in meat; see Table 8.

The characteristic flavor and fragrance components were mostly produced during the cooking process when the flavor precursor molecules reacted with the heme-protein. Gas chromatography—mass spectrometry (GCMS) is a method that combines the features of gas-liquid chromatography and mass spectrometry to separate and identify different substances within a test sample. Samples were evaluated by GCMS to identify the flavor compounds generated after heating and also evaluated for their sensory profiles. Volatile chemicals were isolated from the head space around the flavor reactions. The profile of the volatile chemicals in the headspace around the flavor reaction mixtures is shown in Table 8. In particular, the use of ribose created some additional compounds as compared to glucose, as shown in Table 8.

Notably, the control mixtures of cysteine with ribose or glucose heated in the absence of the LegH heme-protein did not generate the same set of flavor compounds. The flavor reaction mixtures containing LegH also were evaluated by a blinded trained sensory panel, which described the samples with ribose as having beefy, savory, brothy, and gravy-like notes, and the samples with glucose as savory, bloody, metallic, raw meat, and bouillon-like.

TABLE 8

Flavor compounds generated with cysteine, LegH, and either glucose or ribose in the flavor reaction mixture. LegH 1%			
Compounds created	cysteine (10 mM), glucose (20 mM)	cysteine (10 mM), ribose (20 mM)	
35 benzaldehyde	X	X	
2-butanone	X	X	
dimethyl trisulfide	X	X	
2-pentyl-furan	X	X	
2-methyl-propanal	X	X	
thiazole	X	X	
40 butyrolactone	X	X	
2-acetylthiazole	X	X	
pentanal	X	X	
3-methyl-butanol	X	X	
methyl-thiirane	X	X	
nonanal	X	X	
45 heptanal	X	X	
2,3-butanedione	X	X	
1,3,5-cycloheptatriene	X	X	
propyl-cyclopropane	X	X	
2-hexyl-furan	X	X	
butanal	X	X	
2-methyl-butanol	X	X	
50 2-ethyl-furan	X	X	
2-octanone	X	X	
propanal	X	X	
trichloromethane	X		
2-methyl-furan	X	X	
furan	X	X	
55 pyrazine	X	X	
thiophene	X	X	
1,3-dimethyl-benzene	X	X	
octane		X	
octanal	X	X	
thiazole	X	X	
60 2-pentanone		X	
furfural	X	X	
2-nonanone	X	X	
(Z)-2-heptenal	X	X	
(E)-2-heptenal	X	X	
1-octene	X	X	
65 formic acid, heptyl ester	X	X	
2-pentyl-thiophene		X	

TABLE 8-continued

Flavor compounds generated with cysteine, LegH, and either glucose or ribose in the flavor reaction mixture. LegH 1%		
Compounds created	cysteine (10 mM), glucose (20 mM)	cysteine (10 mM), ribose (20 mM)
1-octen-3-one	X	X
3-pentyl-furan	X	X
2-propenal		X
(E)-2-tridecen-1-ol		X
benzene		X
(E)-4-octene		X
1-penten-3-one		X
4-penten-2-one	X	X
2-methyl-thiazole		X
methyl-pyrazine		X
trans-2-(2-pentenyl)furan		X
3-ethylcyclopentanone		X
pyrrole	X	X
2-thiophenecarboxaldehyde		X
3-thiophenecarboxaldehyde		X

Example 5: Heme-Protein in the Presence of Thiamine Affects the Production of Certain Flavor Compounds

The addition of thiamine in a flavor reaction mixtures with a heme protein and other flavor precursors affected the formation of 5-Thiazoleethanol, 4-methyl-furan, 3,3'-dithiobis[2-methyl-thiazole, and 4-methylthiazole. These compounds are known to be present in meat and have beefy, meaty taste notes.

Flavor reaction mixtures at pH 6 containing LegH (1%), cysteine (10 mM), thiamine (1 mM), either glucose or ribose (20 mM), and with or without glutamic acid (10 mM) were prepared and subsequently heated to 150 C for 3 minutes. These flavor reaction samples then were evaluated by GCMS for the flavor compounds generated and evaluated by a trained panel for their sensory profiles. Volatile chemicals were isolated from the head space around the flavor reactions. GCMS showed 4-methyl-5-thiazoleethanol, 3,3'-dithiobis[2-methyl]-furan, and 4-methylthiazole compounds were created by a mixture of LegH with thiamine, a sugar (either glucose or ribose), and cysteine. The same flavor reaction mixtures without thiamine did not generate these compounds; additionally these compounds were not generated when heme-proteins were not present in the flavor reaction mixtures.

The flavor reaction samples also were evaluated by a blinded trained sensory panel, which described the samples with the addition of thiamine as more complex in taste and more beefy, meaty, and savory.

Example 6: Heme-Proteins with Nucleotides Controls Particular Flavor Compound Production

The addition of inosine monophosphate and guanosine monophosphate in mixes with heme protein and other precursors controlled the formation of flavor compounds (E)-4-octene, 2-ethyl-furan, 2-pentanone, 2,3-butanedione, 2-methyl-thiazole, methyl-pyrazine, tridecane, (E)-2-octenal, 2-thiophenecarboxaldehyde, and 3-thiophenecarboxaldehyde. These compounds are known to be present in meat and have a beefy, meaty, buttery, and or savory flavor notes.

Reactions containing heme protein at 1% (LegH) with cysteine (10 mM), and glucose (20 mM), 1 mM IMP and 1 mM GMP, at pH 6.0 were prepared and heated to 150 C for

3 minutes. Characteristic flavor and fragrance components were mostly produced during the cooking process where precursors reacting heme-protein. These samples were evaluated by GCMS for the flavor compounds generated and evaluated for the sensory experience. Volatile chemicals were isolated from the head space around the flavor reaction and identified using GCMS, creating a profile of the volatile chemicals in the headspace around the flavor reaction mixture. GCMS showed 4-octene, 2-ethyl furan, 2-pentanone, 2,3-butanedione, 2-methyl-thiazole, methyl-pyrazine, tridecane, 2-octenal, 2-thiophenecarboxaldehyde, 3-thiophenecarboxaldehyde compounds were created by a mixture of heme-protein LegH with IMP, GMP, glucose, and cysteine. The same samples without IMP and GMP did not generate these compounds, additionally these compounds were also not created when heme-proteins were not present, just precursor molecules. Sensory evaluation by blinded trained panelist found the samples with the addition of inosine and guanosine as described as having more complexity in taste and more beefy, meaty, brothy and savory. FIG. 2 shows the abundance of the novel flavor compounds created with heme protein at 1% was mixed in a reaction at pH 6, with cysteine (10 mM), and glucose (20 mM), IMP (1 mM) and GMP (1 mM), and detected by solid phase microextraction (SPME) and then detected by GCMS.

Example 7: Flavor Generation with the Addition of a Particular Organic Acid

The addition of lactic acid in mixes with heme protein, ribose, and cysteine controlled the formation of the flavor compounds nonanal, thiazole, 2-acetylthiazole, and 8-methyl-1-undecene. These compounds are known to be present in meat.

Reactions containing heme protein at 1%, cysteine (10 mM), and ribose (20 mM), and lactic acid (1 mM), pH 6.0, were prepared and heated to 150 C for 3 minutes. Characteristic flavor and fragrance components were mostly produced during the cooking process where precursors reacting heme-protein. These samples were evaluated by GCMS for the flavor compounds generated and evaluated for the sensory experience. Volatile chemicals were isolated from the head space around the flavor reaction and identified using GCMS, creating a profile of the generated compounds. Nonanal, thiazole, 2-acetylthiazole, and 8-methyl-1-undecene compounds were created by a mixture of LegH with lactic acid, ribose, and cysteine. The same samples without lactic acid did not generate these compounds, additionally these compounds were not created in the absence of heme-proteins.

Sensory evaluation by blinded trained panelist found the samples with the addition of lactic acid as described as beefy, savory, browned, bready, and having malty notes. The sample with everything but lactic acid rated lower in browned, bready and malty notes.

Example 8: Flavor Generated with the Addition of a Particular Amino Acid

The addition of lysine in mixes with heme protein ribose, and cysteine controlled the formation of flavor compounds dimethyl trisulfide, nonanal, 2-pentyl-thiophene, furfural, 2-nonenal, 1-octanol, 2-nonenal, thiazole, 2-acetylthiazole, phenylacetaldehyde, 2-acetylthiazole. These compounds are known to be present in meat and some have a beefy, meaty, and or savory flavor.

25

Reactions containing heme protein at 1%, cysteine (10 mM), and ribose (20 mM), and lysine (1 mM), at pH 6.0, were prepared and heated to 150 C for 3 minutes. These samples were evaluated by GCMS for the flavor compounds generated and evaluated for the sensory experience. Characteristic flavor and fragrance components were mostly produced during the cooking process where precursors could react with the heme-protein. These samples were evaluated by GCMS for the flavor compounds generated and evaluated for the sensory experience. Volatile chemicals were isolated from the head space around the flavor reaction. Dimethyl trisulfide, nonanal, 2-pentyl-thiophene, furfural, 2-nonenal, 1-octanol, 2-nonenal, thiazole, 2-acetylthiazole, phenylacetaldehyde, 2-acetylthiazole compounds were created by a mixture of LegH with lactic acid, ribose, and cysteine. The same samples without lactic acid did not generate these compounds, additionally these compounds were not created when heme-proteins were not present, just precursor molecules. Sensory evaluation by blinded trained panelist found the samples with the addition of lysine as described as roast beefy, savory, and browned. The addition of lysine increased the roasted browned notes.

Example 9—Flavor Compound Production by
Different Heme-Proteins

The addition of different types of heme-proteins (LegH, Barley, *B. myoglobin*, or *A. aeolicus*) in flavor reaction mixtures containing one or more flavor precursor compounds results in many of the same key meat flavors, including but not limited to 2-pentyl-furan, 2,3-Butanedione, Thiophene, 2-methyl-thiazole, Pyrazine, Furan, Pyrrole, 2-methyl-furan and distinct differences in the flavor compounds, including but not limited to 2-pentyl-thiophene, Nonanal, 2-Nonanone, and 1-Octen-3-one. These differences in flavor compounds can change the overall taste profile. The different types of heme-protein were LegH, Barley, *B. myoglobin*, or *A. aeolicus* used at 1% w/w in a reaction mixed with cysteine (10 mM) and ribose (10 mM) at pH 6. The pre-reaction mixture was heated to 150 C for 3 minutes; this reaction created flavor compounds known to be present in meat; see Table 9. The characteristic flavor and fragrance components are mostly produced during the cooking process where the flavor precursor molecules react with the heme-protein. Samples were evaluated by GCMS to identify the flavor compounds generated after heating and also evaluated for their sensory profiles. Volatile chemicals were isolated from the head space around the flavor reactions. Table 9 shows the similarity and differences in volatile flavor compounds created by the different types of heme-proteins.

TABLE 9

Flavor compounds created by different heme-protein when heated with ribose and cysteine.				
Name	LegH	Barley	B. myoglobin	A. aeolicus
Furan	x	x	x	x
Thiazole	x	x	x	x
benzaldehyde	x	x	x	x
2-acetylthiazole	x	x	x	x
2-methyl-propanal	x	x	x	x
furfural	x	x	x	x
2,3-butanedione	x	x	x	x
2-pentyl-furan	x	x	x	x
2-pentanone	x	x		
pyrazine	x	x	x	x

26

TABLE 9-continued

Flavor compounds created by different heme-protein when heated with ribose and cysteine.				
Name	LegH	Barley	B. myoglobin	A. aeolicus
dimethyl trisulfide	x	x	x	x
3-methyl-butanol	x	x		x
2-methyl-thiazole	x	x	x	x
pentanal	x	x	x	x
1,3,5-cycloheptatriene	x	x	x	x
methacrolein	x	x	x	x
heptanal	x	x	x	x
2-methyl-butanol	x	x		x
isothiazole	x	x	x	x
thiophene	x	x	x	x
propanal	x	x	x	x
2-heptenal	x		x	x
methyl-pyrazine	x	x	x	x
1-octene	x		x	x
butanol	x	x	x	x
2-acetyl-propen-2-ol	x	x	x	x
pyrrole	x	x	x	x
2-methyl-furan	x	x	x	x
nonanal		x	x	x
2-propenal		x	x	x
2-decenal		x	x	x
2-nonanone		x		x
2-octanone		x	x	x
2-tridecen-1-ol,			x	x
2-octanone			x	
2-octenal			x	x
4-methyl-2-heptanone			x	x
octanal			x	x
2-undecenal				x
butyrolactone				x
1-octen-3-one				x
3-methylheptyl acetate				x
2-pentyl-thiophene				x

Example 10—Generation of Meat Flavors from
Different Lipids

Several different samples including oils (canola oil or coconut oil), free fatty acids (FFA) (linoleic acid (C18:2), oleic acid (C18:1), stearic acid (C18:0), or myristic acid (C14:0)) and phospholipids (PL) (beef heart polar lipids extract, Biolipon95 (from Perimond), or NatCholinePC40 (from Perimond)) were tested for their ability to produce beefy flavor in the absence and in the presents of other precursors. Oils, FFAs, and PLs were added to 50 mM potassium phosphate buffer (PPB) pH 6.0 or a Maillard reaction mix (MRM) containing 50 mM potassium phosphate pH 6.0, 5 mM Cysteine, 10 mM Glucose, 0.1 mM Thiamine, and 0.1% (w/v) LegHemoglobin. Lipids in combination with MRM were designed to capture the cross reactions of lipid degradation and Maillard reaction productions while lipids in phosphate buffer functioned as a lipid control. The oils were added at 3% of the total 1 mL volume of solution while FFAs and PLs were added at 1% of the total 1 mL volumes. All samples were cooked at 150° C. for 3 mins, cooled to 50° C. and then analyzed using GCMS (SPME fiber sampling of headspace). After all samples were analyzed by GCMS the caps were removed and samples were smelled by a trained flavor scientist and aromas recorded.

27

TABLE 10

Legend showing components of each sample		
Sample Name	Solution	Additives
MRM_None	Maillard Reaction Mix	None
MRM_Linoelic Acid	Maillard Reaction Mix	1% linoelic acid
MRM_Oleic Acid	Maillard Reaction Mix	1% oleic acid
MRM_C14	Maillard Reaction Mix	1% C14:0 free fatty acid
MRM_C18	Maillard Reaction Mix	1% C18:0 free fatty acid
MRM_Canola	Maillard Reaction Mix	3% Canola Oil
MRM_Coconut	Maillard Reaction Mix	3% Coconut Oil
MRM_BeefHeart	Maillard Reaction Mix	1% Beef Heart Polar Lipids Extract
MRM_Biolipon95	Maillard Reaction Mix	1% Biolipon95 (emulsifier)
MRM_NatCholinePC40	Maillard Reaction Mix	1% NatCholinePC40 (emulsifier)
KPhos6_Linoelic Acid	PPB, pH 6	1% linoelic acid
KPhos6_Oleic Acid	PPB, pH 6	1% oleic acid
KPhos6_C14	PPB, pH 6	1% C14:0 free fatty acid
KPhos6_C18	PPB, pH 6	1% C18:0 free fatty acid
KPhos6_Canola	PPB pH 6	3% Canola Oil
KPhos6_Coconut	PPB, pH 6	3% Coconut Oil
KPhos6_BeefHeart	PPB, pH 6	1% Beef Heart Polar Lipids Extract
KPhos6_Biolipon95	PPB, pH 6	1% Biolipon95 (emulsifier)
KPhos6_NatCholinePC40	PPB, pH 6	1% NatCholinePC40 (emulsifier)

Table 11 contains the aroma descriptions and Table 12 contains the GCMS data from the most interesting samples analyzed. Many of the lipids introduced a “fatty” aroma to MRM that was otherwise absent. The combinations of Linoleic Acid or NatCholinePC40 in MRM produced the greatest abundance of fatty compounds suggesting that these lipids may improve the flavor perception of beef tallow. Linoleic Acid and NatCholinePC40 also showed high abun-

28

dance of earthy-mushroom aromas. The addition of lipids to MRM significantly increased the abundance of “nutty & roasted” aromas. Less desirable “green” aroma compounds were most prominent in samples with unsaturated free fatty acids (linoleic acid or oleic acid) or phospholipids. In general, the addition of lipids significantly increased the number of target beef compounds made.

TABLE 11

Aroma descriptions of each sample after it was cooked.	
Sample Names	Aroma Descriptions
MRM_Only	brothy, malty, beef stew
KPhos6_BeefHeart	fatty, creamy, beef tallow, slight sweet, slight roasted nutty
MRM_BeefHeart	fatty, beef tallow, old meat, mushroom
KPhos6_Biolipon95	fatty, fresh
MRM_Biolipon95	fatty, brothy, hay, malty green
KPhos6_NatCholinePC40	light fatty, fresh
MRM_NatCholinePC40	fatty, beef tallow, brothy
K-Phos6_C14	light/faint plastic/waxy
MRM_C14	brothy, beefy, minty, fresh
K-Phos6_C18	light/faint plastic/waxy
MRM_C18	beefy with cucumber &/or pepper aroma
K-Phos6_Canola	fresh, cucumber
MRM_Canola	fatty, brothy, oil, roasted nuts
K-Phos6_Coconut	nothing
MRM_Coconut	brothy, beefy, slight fatty, crackers
K-Phos6_Oleic Acid	fresh, cucumber, camphorous/minty-like
MRM_OleicAcid	herbal, plastic, slight cheesy, brothy
K-Phos6_Linoelic Acid	light plastic
MRM_Linoelic Acid	fatty, light waxy, brothy, herbal

TABLE 12

List of aromatic compounds found in Beef by GCMS and a chart showing which were detected in each lipid plus MRM sample.				
Compounds in Beef	MRM only	MRM_BeefHeart	MRM_NatCholinePC40	MRM_Linoelic acid
(s)-isopropyl lactate	N	N	N	N
1-ethyl-5-methylcyclopentene	Y	Y	Y	Y
1-heptanol	N	Y	N	N
1-hepten-3-ol	N	Y	Y	Y
1-heptene	N	Y	Y	Y
2-methyl-1-heptene	N	N	N	N
1-hexanol	N	Y	Y	Y
2-ethyl-1-hexanol	N	N	N	N
1-nonanol	N	N	Y	N
1-nonene	N	Y	Y	N
1-octanol	N	Y	Y	N
1-octen-3-ol	N	Y	Y	Y
1-octen-3-one	Y	Y	Y	Y
1-octene	N	N	N	N
1-pentanol	N	Y	Y	Y
1-penten-3-ol	N	Y	Y	N
1-propanol	N	N	N	N
8-methyl-1-undecene	N	Y	Y	Y
1,3-hexadiene	N	N	N	Y
3-ethyl-2-methyl-1,3-hexadiene	N	Y	Y	Y
1,3-octadiene	Y	N	N	Y
1,3,5-cycloheptatriene	N	N	N	N
2,3-dihydro-5,6-dimethyl-1,4-dioxin	N	N	N	N
1,7-octadien-3-ol	N	Y	N	N
1h-pyrrole-2-carboxaldehyde	N	N	N	N

TABLE 12-continued

List of aromatic compounds found in Beef by GCMS and a chart showing which were detected in each lipid plus MRM sample.				
Compounds in Beef	MRM only	MRM_BeefHeart	MRM_NatCholinePC40	MRM_Linoleic acid
2-methyl-1H-pyrrole	N	N	N	N
2-acetyl-2-thiazoline	Y	N	N	N
2-acetylthiazole	Y	Y	Y	Y
2-butanone	N	Y	Y	Y
2-butenal	N	Y	Y	Y
2-ethyl-2-butenal	N	N	N	Y
3-methyl-2-butenal	N	N	Y	Y
3-methyl-2-cyclohexen-1-one	N	N	N	N
2-decanone	Y	Y	Y	N
(E)-2-decenal	N	N	N	N
(Z)-2-decenal	Y	Y	Y	Y
2-furanmethanol	N	N	N	N
2-heptanone	Y	Y	Y	Y
6-methyl-2-heptanone	N	N	Y	N
(E)-2-heptenal	N	Y	Y	Y
(Z)-2-heptenal	N	N	N	Y
(E)-2-hexenal	N	Y	Y	Y
2-ethyl-2-hexenal	N	N	N	N
2-methyl-2-heptene	Y	N	N	N
2-n-heptylfuran	Y	N	N	N
2-n-octylfuran	Y	Y	Y	N
2-nonanone	N	Y	Y	N
(E)-2-nonenal	Y	Y	Y	Y
(Z)-2-nonenal	N	N	N	Y
2-octanone	Y	Y	Y	Y
(Z)-2-octen-1-ol	Y	Y	Y	Y
(E)-2-octenal	N	Y	Y	Y
2-pentanone	N	Y	Y	N
1-propoxy-2-propanol	N	N	N	N
1-(acetyloxy)-2-propanone	Y	N	N	N
1-hydroxy-2-propanone	Y	N	N	N
2-propenal	N	N	N	Y
2-thiophenecarboxaldehyde	Y	Y	Y	Y
2-undecenal	N	Y	Y	Y
2,3-butanedione	N	N	N	Y
2,3-pentanedione	N	N	N	N
(E,E)-2,4-decadienal	N	Y	Y	Y
2,4-decadienal	N	N	N	Y
(E,E)-2,4-heptadienal	N	Y	Y	Y
(E,E)-2,4-nonadienal	N	Y	Y	Y
2,6-dimethylpyrazine	N	N	N	N
(E,Z)-2,6-nonadienal	N	N	Y	N
5-ethyl-2,3-dihydro-2H-furanone	N	Y	Y	Y
5-methyl-2(3H)-furanone	N	N	N	N
dihydro-5-pentyl-2(3H)-furanone	N	N	Y	Y
dihydro-5-propyl-2(3H)-furanone	N	N	N	N
2(5H)-furanone	N	N	N	N
tetrahydro-6-methyl-2H-pyran-2-one	N	N	N	N
3-ethylcyclopentanone	N	Y	Y	Y
3-hexanone	N	N	N	N
3-methyl-2-thiophenecarboxaldehyde	N	N	N	N
3-octanone	Y	Y	N	Y
3-octen-2-one	N	Y	Y	Y
3-thiophenecarboxaldehyde	N	Y	Y	Y
(E,E)-3,5-octadien-2-one	N	N	Y	Y
dihydro-2-methyl-3(2H)-furanone	N	N	N	N
4-cyanocyclohexene	N	N	N	N
4-cyclopentene-1,3-dione	N	N	Y	N
4-decyne	N	Y	N	N
(Z)-4-heptenal	N	Y	Y	Y
4-methyloctanoic acid	N	N	N	N
(E)-4-octene	N	N	N	N
2,3-dihydro-3,5-dihydroxy-6-methyl-4(H)-pyran-4-one	Y	N	N	N
6-methyl-5-hepten-2-one	Y	N	N	N
acetaldehyde	N	N	N	Y
acetic acid	N	N	N	N
acetic acid ethenyl ester	Y	N	N	N

TABLE 12-continued

List of aromatic compounds found in Beef by GCMS and a chart showing which were detected in each lipid plus MRM sample.				
Compounds in Beef	MRM only	MRM_BeefHeart	MRM_NatCholinePC40	MRM_Linoleic acid
acetoin	Y	N	N	N
acetone	Y	N	N	Y
acetonitrile	N	N	N	Y
benzaldehyde	Y	Y	Y	Y
4-ethyl-benzaldehyde	N	Y	Y	N
benzene	Y	N	N	N
benzoic acid, hydrazide	Y	N	N	N
butanal	Y	N	N	Y
2-methyl-butanal	N	N	N	N
3-methyl-butanal	Y	N	N	N
butanoic acid	N	N	N	N
butyrolactone	Y	Y	N	Y
caprolactam	N	N	N	N
carbon disulfide	N	N	N	Y
1-ethyl-1-methyl- cyclopentane	Y	Y	Y	Y
propyl-cyclopropane	N	N	Y	Y
decanal	N	Y	Y	N
dihydro-3-(2H)-thiophenone	N	N	N	N
Dimethyl sulfide	Y	N	N	N
dimethyl sulfone	N	N	N	N
dimethyl trisulfide	Y	Y	N	N
ethanethiol	N	N	N	N
ethanol	N	N	N	Y
1-(1H)-pyrrol-2-yl)- ethanone	N	N	N	N
1-(2-furanyl)-ethanone	N	N	N	N
ethosuximide	Y	N	N	N
formic acid, heptyl ester	Y	Y	N	N
furan	Y	N	N	Y
2-ethyl-furan	Y	N	N	N
2-hexyl-furan	Y	N	N	Y
2-methyl-furan	N	N	N	Y
2-pentyl-furan	N	Y	Y	Y
2-propyl-furan	N	N	Y	Y
3-methyl-furan	Y	N	N	N
3-pentyl-furan	Y	Y	Y	Y
furfural	N	Y	Y	Y
heptanal	N	Y	Y	Y
heptanoic acid	N	N	N	Y
2-methyl-hex-2-yn-4-one	N	N	N	N
hexanoic acid	N	N	N	Y
hydrogen sulfide	N	N	N	N
m-aminophenylacetylene	N	N	N	N
maleic anhydride	N	N	N	N
methacrolein	N	N	N	N
methanethiol	N	N	N	N
methyl ethanoate	N	N	N	N
methyl isobutyl ketone	Y	N	N	N
n-caproic acid vinyl ester	N	Y	Y	N
nonanal	N	Y	Y	Y
3-methyl-nonane	Y	N	N	N
nonanoic acid	Y	N	N	N
octanal	N	Y	Y	Y
octane	N	N	N	Y
octanoic acid	N	N	N	Y
oxalic acid, isobutyl pentyl ester	Y	N	N	N
p-cresol	N	N	N	N
pentanal	N	N	N	Y
pentanoic acid	Y	N	N	Y
4-ethyl-phenol	N	Y	Y	N
phenylacetaldehyde	Y	Y	Y	Y
(p-hydroxyphenyl)- phosphonic acid	Y	N	N	N
propanal	N	N	N	Y
2-methyl-propanal	N	N	N	N
propanoic acid	N	N	N	N
2-methyl-propanoic acid	Y	N	N	N
propanoic acid, ethenyl ester	N	N	N	N
pyrazine	N	Y	N	Y
2-ethyl-5-methyl-pyrazine	N	N	N	N
2-ethyl-6-methyl-pyrazine	N	N	N	N
2,3-dimethyl-pyrazine	N	N	N	N

TABLE 12-continued

List of aromatic compounds found in Beef by GCMS and a chart showing which were detected in each lipid plus MRM sample.				
Compounds in Beef	MRM only	MRM_BeefHeart	MRM_NatCholinePC40	MRM_Linoleic acid
2,5-dimethyl-pyrazine	N	N	N	N
3-ethyl-2,5-dimethyl-pyrazine	Y	N	N	N
ethyl-pyrazine	N	N	N	N
methyl-pyrazine	N	N	N	N
trimethyl-pyrazine	Y	N	N	N
pyridine	Y	N	Y	N
pyrrole	Y	Y	Y	Y
styrene	Y	N	Y	N
thiazole	Y	Y	Y	Y
methyl-thiirane	N	N	N	N
thiophene	N	N	N	Y
2-hexyl-thiophene	Y	N	Y	N
2-pentyl-thiophene	N	Y	N	N
trans-2-(2-pentenyl)furan	N	Y	Y	N
trans-3 --nonen-2-one	N	Y	Y	Y
undecanoic acid	N	N	N	N
Total # of Compounds Detected:	54	63	66	76

In samples having fatty or creamy aromas, 2,4-decadienal, (E,E)-2,4-nonadienal, (E,E)-2,4-heptadienal, and/or (E,E)-2,4-decadienal were detected in the KPhos6_BeefHeart, MRM_BeefHeart, MRM_BioLipon95, MRM_NatCholinePC40, KPhos6_Canola, MRM_Canola, KPhos6_Oleic Acid, KPhos6_Linoleic acid and MRM_Linoleic acid samples. For (E,E)-2,4-decadienal, the strongest signal intensity was in the MRM_NatCholinePC40 sample, followed by the MRM_Linoleic acid, KPhos6_Linoleic acid, MRM_BeefHeart, MRM_BioLipon95, KPhos6_BeefHeart, MRM_Oleic Acid, and KPhos6_Oleic Acid samples. For (E,E)-2,4-heptadienal, the strongest signal intensity was in the MRM_NatCholinePC40 sample followed by the MRM_Canola sample. (E,E)-2,4-heptadienal also was detected in the MRM_BioLipon95, MRM_BeefHeart, and MRM_Linoleic acid samples. For (E,E)-2,4-nonadienal, the strongest signal intensity was in the MRM_Canola and MRM_Linoleic acid samples. (E,E)-2,4-nonadienal also was detected in the KPhos6_Canola, MRM_NatCholinePC40, MRM_BioLipon95, MRM_BeefHeart, and KPhos6_Linoleic acid samples. For 2,4-decadienal, the strongest signal intensity was in the MRM_Linoleic acid sample. 2,4-decadienal also was detected in KPhos6_Linoleic acid, MRM_Canola, and KPhos6_Oleic Acid samples.

In samples having earthy or mushroom aromas, 3-octen-2-one, 1-octen-3-one, 3-octanone, and/or 1-octen-3-ol were detected in the KPhos6_BeefHeart, MRM_BeefHeart, KPhos_BioLipon95, MRM_BioLipon95, KPhos_NatCholinePC40, MRM_NatCholinePC40, MRM_Canola, KPhos6_Oleic Acid, MRM_Oleic Acid, KPhos6_Linoleic acid, and MRM_Linoleic acid samples. For 1-octen-3-ol, the strongest signal intensity was in the MRM_Linoleic acid sample, followed by MRM_NatCholinePC40, KPhos6_Linoleic acid, MRM_BeefHeart, KPhos6_BeefHeart, MRM_Canola, MRM_BioLipon95, KPhos6_Oleic Acid, and MRM_Oleic Acid samples. 3-octanone was detected in the MRM_Oleic Acid, KPhos6_Linoleic acid, and MRM_Linoleic acid samples. For 1-octen-3-one, the strongest signal intensity was in the MRM_Linoleic acid and MRM_BeefHeart samples, followed by KPhos6_Linoleic acid, MRM_NatCholinePC40, KPhos6_BeefHeart, MRM_

BioLipon95, MRM_Oleic Acid, and KPhos6_Oleic Acid samples. For 3-octen-2-one, the strongest signal intensity was in the KPhos6_Linoleic acid sample, followed by MRM_Linoleic acid, MRM_NatCholinePC40, KPhos6_BeefHeart, KPhos6_Oleic Acid, MRM_Oleic Acid, MRM_BeefHeart, MRM_BioLipon95, MRM_Canola, KPhos_BioLipon95, and KPhos_NatCholinePC40. Pyrazine was detected in the MRM_Coconut, MRM_C18, MRM_C14, and MRM_BioLipon95 samples.

In samples having a nutty and roasted aroma, thiazole and 2-acetylthiazole were the most abundant compounds detected, along with pyrazine, methyl pyrazine, trimethyl pyrazine, and 3-ethyl-2,5-dimethylpyrazine. 2-acetylthiazole was detected in all samples with MRM and most abundant in samples with MRM_BeefHeart, MRM_BioLipon95, MRM_Canola, and MRM_Coconut. Thiazole was created in samples with MRM_Coconut, MRM_BeefHeart, MRM_BioLipon95, MRM_C14, MRM_C18, MRM_Canola, MRM_Oleic acid and MRM_Linoleic acid and MRM_NatCholinePC40. Pyrazine was present in the largest amount in samples with MRM_Coconut, followed by samples MRM_BeefHeart, MRM_BioLipon95, MRM_C14, MRM_C18, MRM_Canola having roughly equal amount, MRM_Oleic acid and MRM_Linoleic acid sample had even less. Methyl-pyrazine was present in MRM_BioLipon95 and MRM_Coconut. 3-ethyl-2,5-dimethyl-pyrazine and trimethyl-pyrazine, were present only without phospholipids in the MRM.

In samples having green, vegetable, or grass aromas, 1-heptanol, 1-hepten-3-ol, 1-hexanol, (E)-2-heptenal, (Z)-2-heptenal, (E)-2-hexenal, 2-pentyl-furan, and/or heptanal were detected in the KPhos6_BeefHeart, MRM_BeefHeart, KPhos_BioLipon95, MRM_BioLipon95, KPhos_NatCholinePC40, MRM_NatCholinePC40, KPhos_C14, MRM_C14, KPhos_C18, MRM_C18, MRM_Canola, MRM_Coconut, KPhos6_Oleic Acid, MRM_Oleic Acid, KPhos6_Linoleic acid, and MRM_Linoleic acid samples. For 2-pentyl-furan, the strongest signal intensity was in the KPhos6_BeefHeart sample, followed by the KPhos6_Linoleic acid, MRM_BioLipon95, MRM_Linoleic acid, MRM_BeefHeart, MRM_Oleic Acid, MRM_NatCholinePC40, MRM_Canola, KPhos6_Oleic Acid, and KPhos_

35

NatCholinePC40 samples. For (E)-2-heptenal, the strongest signal intensity was in the MRM_BeefHeart, MRM_Canola, MRM_Oleic Acid, and KPhos6_Linoleic acid samples, followed by the KPhos6_Oleic Acid, MRM_BioLipon95, KPhos6_BeefHeart, MRM_Linoleic acid, MRM_NatCholinePC40, Kphos_BioLipon95, and Kphos_NatCholinePC40 samples. For (Z)-2-heptenal, the strongest signal intensity was in the MRM_Linoleic acid sample. MRM_Linoleic acid also was detected in the KPhos6_Linoleic acid sample. For heptanal, the strongest signal intensity was in the MRM_Oleic Acid sample, followed by the KPhos6_Oleic Acid, MRM_C14, MRM_C18, MRM_Canola, MRM_BeefHeart, MRM_NatCholinePC40, MRM_Linoleic acid, and KPhos6 BeefHeart samples. For, (E)-2-hexenal, the strongest signal intensity was in the MRM_Linoleic acid sample, followed by the MRM_NatCholinePC40, KPhos6_Linoleic acid, and MRM_Oleic Acid samples.

Example 11—Creation of Beefy Flavors Using Complex Precursor Mixtures

A formulation was prepared (the “magic mix,” see Table 13 containing the estimated concentrations of amino acids, sugars, and other small molecules in beef based on their values reported in literature. The magic mix was tested for its ability to produce beefy flavors in the presence of LegHemoglobin (LegH). The magic mix and 1% w/v LegH were added to the meat replica, pH 6.0 (see Table 4) and baked in a convection oven for 7 minutes at 160° C. A control sample was prepared by adding 1% w/v LegH to the meat replica, pH 6.0 and baking in a convection oven for 7 minutes at 160° C.

The meat replica sample containing only LegH, was compared to the meat replica sample containing the magic mix and LegH by a sensory panel and GCMS analysis. Five tasters rated the flavored meat replicas for beefiness, bitterness, and levels of savory flavors, and off flavors. Each property was rated on a 7 point scale in which 7 was the highest amount of the specified property (e.g., a standard 80:20 ground beef would be rated 7 on the beefy scale). The Magic Mix flavor was rated one point higher in beefy character than the LegH only sample (FIG. 1).

To determine which chemical products were produced upon heating, a solution of Magic Mix was prepared with 1% w/v LegH at pH 6.0. The samples were cooked with shaking at 150° C. for three minutes, then Solid Phase Micro Extraction (SPME) was performed for twelve minutes at 50° C. to extract the volatile compounds above the headspace of the reaction. A search algorithm was used to analyze the retention time and mass fingerprint information of the volatile compounds and assign chemical names to peaks. Table 14 shows the compounds identified in both the Magic Mix+LegH (MM, average of two samples) and in the LegH alone in buffer (LegH, average of five samples) samples. The compounds in Table 14 are listed in order of the retention time (R.T., in seconds), and are designated as having a zero peak area (0), or a small (S), medium (M), or large (L) average peak area. Hundreds of compounds were identified between the samples, many of which are characteristic of beefy aroma, including but not limited to 1,3-bis(1,1-dimethylethyl)-benzene, 2-methyl 3-furanthiol, and Bis(2-methyl-4,5-dihydro-3-furyl)disulfide, which increased in the samples containing the Magic Mix and LegH.

36

TABLE 13

Chemical entities added to the Magic Mix		
	Chemical entity	mM
5	Alanine	5.6
	Arginine	0.6
	Asparagine	0.8
	Aspartate	0.8
	Cysteine	0.8
10	Glutamic acid	3.4
	Glutamine	0.7
	Glycine	1.3
	Histidine	0.6
	Isoleucine	0.8
15	Leucine	0.8
	Lysine	0.7
	Methionine	0.7
	Phenylalanine	0.6
	Proline	0.9
20	Threonine	0.8
	Tryptophan	0.5
	Tyrosine	0.6
	Valine	0.9
	glucose	5.6
25	Ribose	6.7
	Maltodextrin	5.0
	Thiamine	0.5
	GMP	0.24
	IMP	0.6
30	Lactic acid	1.0
	creatine	1.0
	NaCl	10
	KCl	10
	Kphos pH 6.0	10

TABLE 14

Compounds identified with GC-MS analysis in samples with MM and LegH, or LegH alone (average of five samples)			
R.T.(s)	Name	MM with LegH	LegH alone
40	248 acetaldehyde	L	S
	256.3 carbon disulfide	L	S
	264.3 dimethyl sulfide	S	0
	265 oxalic acid, isobutyl pentyl ester	M	0
	268.1 2,3,4-trimethyl-pentane	M	0
45	269.2 methanethiol	S	0
	283.4 propanal	M	0
	285.4 octane	M	0
	287.1 furan	M	0
	295.3 2-methyl-propanal	L	S
50	297.6 acetone	L	S
	319.3 2-propenal	M	S
	338.1 2-methyl-furan	M	S
	342.1 butanal	L	S
	344.2 2,4-dimethyl-1-heptene	M	0
55	346.3 methacrolein	M	0
	357.4 methyl-thiirane	L	0
	360.2 3-methyl-furan	S	0
	363.7 butanone	L	S
	368.9 2,3-dihydro-5-methyl-furan	M	S
60	376.4 2-methyl-butanol	L	M
	381.1 3-methyl-butanol	L	M
	390.6 isopropyl alcohol	0	S
	399.6 ethanol	L	M
	406.2 2-propenoic acid, methyl ester	M	0
65	408.2 benzene	S	0
	414.4 methyl vinyl ketone	M	0
	416.4 2,2,4,6,6-pentamethyl-heptane	M	0
	422.6 2-ethyl-furan	S	0
	438.4 2-ethylacrolein	M	0
65	449.9 2-pentanone	S	0
	453.2 pentanal/2,3-butanedione	L	0
	453.8 2,3-butanedione	L	M

TABLE 14-continued

Compounds identified with GC-MS analysis in samples with MM and LegH, or LegH alone (average of five samples)			
R.T.(s)	Name	MM with LegH	LegH alone
472.8	4,7-dimethyl-undecane	M	S
485.9	2-methyl-pentanal	M	0
492.6	2-methyl-1-penten-1-one	S	0
496.6	(E)-3-penten-2-one	M	0
508.6	1-penten-3-one	M	0
510.6	trichloromethane	M	M
520.4	p-dithiane-2,5-diol	M	0
525.5	3-methyl-pentanal	M	0
535.1	(E)-5-decene	M	0
536.5	toluene	M	S
537.9	2-butenal	M	S
543.8	4-penten-2-one	M	0
550.8	methyl thiolacetate	M	0
683.7	p-xylene	S	0
727.4	dimethyl selenone	M	0
738.3	methyl isopropyl disulphide	M	0
755	2-heptanone	M	0
758.7	heptanal	L	0
781.9	1,3-diisopropoxy-1,3-dimethyl-1,3-disilacyclobutane	S	M
789.4	3-methyl-2-butenal	M	0
793.4	4-methyl-2-heptanone	M	0
810.4	pyrazine	M	0
818.8	isothiazole	S	0
827.1	acetyl valeryl	M	0
831.8	2-pentyl-furan	L	0
851	2-methyl-thiazole	S	0
853.3	isothiocyanato-methane	S	0
870.9	thiazole	L	0
879.2	styrene	M	0
890.7	1-(methylthio)-propane	M	0
895.6	methyl-pyrazine	M	0
910.5	thiocyanic acid, methyl ester	S	0
918.6	4-methylthiazole	M	0
921.4	2-octanone	M	0
923.9	2-methyl-cyclopentanone	M	0
927.9	octanal	L	S
934.3	tridecane	M	0
948.8	trans-2-(2-pentenyl)furan	S	0
961.9	1-hydroxy-2-propanone	M	0
974.5	(E)-2-heptenal	M	0
987.4	5-methyl-1-undecene	M	0
993.8	2-hexyl-furan	M	0
1007.8	7-methyl-(E)-5-undecene	M	0
1024.1	2-methyl-5-(methylthio)-furan,	S	0
1058.6	2-butyl-1-decene	M	0
1079.3	dimethyl trisulfide	L	S
1085.3	2-nonanone	M	0
1093.2	nonanal	L	M
1142.3	1,3-bis(1,1-dimethylethyl)-benzene	M	0
1149.6	(E)-2-octenal	M	0
1164.5	1-heptanol	M	0
1193.5	methional	L	0
1198.8	acetic acid	M	S
1207.2	furfural	M	0
1242.1	2-decanone	M	0
1250.8	decanal	M	0
1265.2	1-decen-3-one	M	0
1283.3	pyrrole	M	0
1292.6	5-ethenyl-4-methyl-thiazole	M	0
1294.3	benzaldehyde	L	M
1303.7	2-n-octylfuran	M	0
1305.6	(E)-2-nonenal	M	0
1341.4	1-octanol	M	0
1361.1	2-methyl-1(H)-pyrrole	S	0
1391.7	2-undecanone	M	0
1401.2	(E)-2-octen-1-ol	M	0
1448	butyrolactone	S	S
1456.3	(E)-2-decenal	M	0
1462.4	phenylacetaldehyde	L	S
1466.3	2-acetylthiazole	L	0
1471.3	acetophenone	M	S
1475.4	1-nonanol	M	0

TABLE 14-continued

Compounds identified with GC-MS analysis in samples with MM and LegH, or LegH alone (average of five samples)			
R.T.(s)	Name	MM with LegH	LegH alone
1487	methyl (methylthio)methyl disulfide	M	0
1497.1	5-(2-chloroethyl)-4-methylthiazole	L	0
1497.5	1-(ethylthio)-2-(methylthio)-buta-1,3-diene	L	S
1512	3-thiophenecarboxaldehyde	M	0
1518.8	2-nonen-4-one	M	0
1531.7	2-thiophenecarboxaldehyde	S	0
1543.9	dodecanal	M	0
1551.6	4-ethyl-2-methyl-pyrrole	S	0
1558.2	3-(methylthio)-propanenitrile	S	0
1561.2	3-decen-2-one	M	0
1613.1	bis(2-methyl-4,5-dihydro-3-furyl) disulfide	M	0
1615.6	1,10-undecadiene	M	0
1619.5	2-undecenal	S	0
1668.9	2-phenylpropenal	M	0
1692.3	(Z)-3-decen-1-ol, acetate	M	0
1733.1	3-phenyl-furan	S	0
1739.7	4-nitrophenyl 2-thiophenecarboxylic acid ester	S	0
1741.2	5-formyl-4-methylthiazole	M	0
1749.7	pentanoic acid, 2,2,4-trimethyl-3-hydroxy-, isobutyl ester	M	0
1765.5	benzyl alcohol	S	0
1774.2	pentanoic acid, 2,2,4-trimethyl-3-hydroxy-, isobutyl ester	S	0
1796.9	dodecanal	M	0
1806.1	(1-ethyl-1-propenyl)-benzene	S	0
1825.6	1-undecanol	M	S
1827.9	2-methyl-3-furanthiol	M	0
1828.3	2-methyl-3-(methylthio) furan	M	0
1836.1	4-chloro-2,6-bis(1,1-dimethylethyl)-phenol	S	0
1844.1	phenol	S	S
1845.3	[(methylsulfonyl)methyl]-benzene	S	0
1850.3	(e)-2-tridecen-1-ol	M	0
1859.9	1-heptyl-1,2,3,4-tetrahydro-4-methyl-naphthalene	S	0
1863.2	2,4-decadienal	S	0
1905.1	3,3'-dithiobis[2-methyl]-furan	M	0
1906.9	3,5-di-tert-butylbenzoic acid	S	0
1909.6	4-ethoxy-benzoic acid, ethyl ester	S	0
1921.5	3-(phenylmethyl)-2,5-piperazinedione	S	0
1944.5	9-octadecenal	M	0
1959.7	3,5-bis(1,1-dimethylethyl)-phenol	M	S
1968.4	4-methyl-5-thiazoleethanol	M	S
2007.8	1,1'-(1,2-cyclobutanediyl)bis-cis-benzene	S	0
2019.8	benzoic acid	S	S
2026.4	4-quinolinecarboxaldehyde	S	0
2027.8	m-aminophenylacetylene	M	0

Example 12—Ferrous Chlorin Catalyzes Production of Meat-Like Flavor Compounds

Fresh green spinach (10 lb) was added to 500 mL water and finely ground in a Vitamix blender to yield 2 L of green suspension. Acetone (8 L) was added with mixing and the material was allowed to extract for 1 hour. The material was filtered through Whatman filter paper and the acetone was removed on a rotary evaporator (Buchi). To the residual green suspension (500 mL) was added 2 mL of 10 M HCl, causing the suspension to turn brown. To this was added 1 g of FeCl₂·4H₂O in 10 mL H₂O. The solution was shaken then left at 4° C. for 16 hours. This suspension was extracted with diethyl ether (3×50 mL) to give a bright green organic phase, the combined organics were washed with saturated sodium chloride solution, dried over sodium sulfate, filtered and evaporated to leave a black paste (1.1 g). The pellet was dissolved in chloroform for fractionation.

Chlorophyll and Ferrous chlorin crude fractions were stored at -20° C. Crude extracts were fractionated by

39

reverse-phase high-pressure liquid chromatography (RP-HPLC). HPLC conditions are outlined in Table 15. Both chlorophyll and ferrous chlorophyll were eluted from the column with a peak retention time of 7.6 minutes. Eluted material was collected from 7.3-8.0 minutes. Collected fractions were pooled and stored on ice. Collected fractions were re-chromatographed and showed a single peak with retention time 7.6 minutes. The desired fractions were pooled, then 10% sunflower oil was added, methanol was removed on a rotary evaporator (Buchi).

TABLE 15

HPLC conditions for purification of chlorophyll and ferrous chlorin from crude extract.	
Sample:	Chlorophyll or Fe-chlorin (~2 mg/mL in CHCl ₃)
System:	Agilent 1100 with Chemstation
Column:	Zorbax Bonus-RP (4.6 × 250 mm, 5 μm)
Mobile phase:	acetonitrile, methanol, ethyl acetate (60:20:20) isocratic flow
Temperature:	30° C.
Flow Rate:	1.0 mL per minute
Injection volume:	0.05 mL

Preparation of Flavor Reaction Containing Ferrous Chlorin or Leghemoglobin

A solution of ferrous chlorophyll was mixed with the Magic Mix (Table 13) to a final concentration of 0.35% ferrous chlorin, 1% glycerol, 0.005% tween-20, 5% sunflower oil, 100 mM NaCl, 20 mM phosphate at pH 6. Leghemoglobin (0.35%) at pH 6 in phosphate buffer (20 mM), 100 mM NaCl, was mixed with the Magic Mix (Table 13), 1% glycerol, and 0.005% tween-20. The flavor reaction mixtures were heated to 150° C. for 3 minutes; this reaction created flavor compounds known to be present in meat, created by hemoglobin and also created by ferrous chlorin; see Table 16.

The characteristic flavor and fragrance components were mostly produced during the cooking process when the flavor precursor molecules reacted with the heme-protein or the ferrous chlorophyll. Samples were evaluated by GCMS to identify the flavor compounds generated after heating. Volatile chemicals were isolated from the headspace around the flavor reactions. The profile of the volatile chemicals in the headspace around the flavor reaction mixtures that were similar between heme-protein and ferrous chlorin are shown in Table 16. Notably, many of the compounds created by the ferrous chlorin are important in the flavor of meat.

TABLE 16

Flavor Compounds created by both Ferrous Chlorin and LegH with Magic Mix.	
1-heptanol	acetone
1-hexanol	acetonitrile
1-octanol	benzaldehyde
1-octen-3-ol	butanal
1-octen-3-one	2-methyl-butanol
1-pentanol	dimethyl trisulfide
2-acetylthiazole	ethyl acetate
2-butanal	furan
3-methyl-2-butanal,	2-ethyl-furan
(Z)-2-decenal	2-hexyl furan
6-methyl-2-heptanone	2-pentyl-furan
(E)-2-heptenal	furfural
(E)-2-hexenal	heptanal
2-methyl-3-furanthiol	aminophenylacetylene
(E)-2-nonenal	methacrolein
(E)-2-octenal	methional
2-pentanone	octanal

40

TABLE 16-continued

Flavor Compounds created by both Ferrous Chlorin and LegH with Magic Mix.	
1-hydroxy-2-propanone	octane
2-thiophenecarboxaldehyde	oxalic acid, diallyl ester
2-undecenal	2,3-butanedione
3-methyl-3-buten-2-one	2-methyl-propanal
3-thiophenecarboxaldehyde	pyrazine
(E)-4-octene,	2,3-dimethyl-pyrazine
methyl-pyrazine	2,5-dimethyl-pyrazine
thiazole	

Example 13—Flavor Creation by Immobilized Hemin

Preparation of Hemin Linked CM Sepharose.

200 mg of bovine hemin (Sigma Aldrich) was loaded into a scintillation vial. A small magnetic stir bar, 800 μL acetonitrile, 64 μL 4-methylmorpholine, and 71 mg of N-hydroxysuccinimide were added in that order. The vial was placed in an ice bath and chilled then 118 mg of N-(3-dimethylaminopropyl)-N'-ethyl-carbodiimide hydrochloride was added with stirring, followed by 845 μL of Jeffamine ED900. This was stirred while allowing the black mixture to warm to ambient temperature. Chloroform (10 mL) was added to the mixture followed by water (4 mL). A flashlight was used to distinguish between organic and aqueous layers since both were black and the organic layer was pipetted off and concentrated to a dark black oil. The oil was dissolved in a 4:1 mixture of acetonitrile and ethanol to make an approximately 10% strength solution that was inky black in color.

2 mL of water swelled and equilibrated CM Sepharose was equilibrated in a BioRad minicolumn with 3 volumes of acetonitrile. The resin was resuspended in 1 mL acetonitrile and pipetted into a scintillation vial. This was followed with 44 microliters 4-methylmorpholine, 23 mg N-hydroxysuccinimide, and 39 mg of solid N-(3-dimethylaminopropyl)-N'-ethyl-carbodiimide hydrochloride. The mixture was vortexed vigorously and then shaken for three hours. To this white solid was added 570 microliters of inky black 20% strength hemin coupled diamine. The black solid was vortexed and shaken for an hour. The slurry strongly resembled Turkish coffee. The mixture was poured into a BioRad minicolumn and filtered, washed with acetonitrile until what came out no longer resembled espresso, then switched to deionized water, and finally 20 mM pH 9 sodium carbonate buffer. The black solid was washed until the effluent ran clear and then resuspended in 2 mL of buffer for storage until use.

Flavor Reaction

The flavor reaction was created with heme protein (equine myoglobin-Sigma) at 0.35% in a phosphate buffer (20 mM) at pH 6.0 with 100 mM NaCl, this was mixed with Magic Mix (Table 13). Another flavor reaction was created with Immobilized Hemin at 0.35% in a phosphate buffer (20 mM) at pH 6.0 with 100 mM NaCl, this was mixed with Magic Mix (Table 13). The flavor reaction mixtures were heated to 150° C. for 3 minutes; this reaction created flavor compounds known to be present in meat.

The characteristic flavor and fragrance components were mostly produced during the cooking process when the flavor precursor molecules reacted with the Heme-protein or the immobilized Hemin. Samples were evaluated by GCMS to identify the flavor compounds generated after heating. Volatile chemicals were isolated from the headspace around the flavor reactions. As can be seen in Table 17, immobilized

41

hemin catalyzed production of compounds similar to those whose production was catalyzed by myoglobin free in solution. Notably, the profiles of flavor compounds, measured by GCMS, produced by cooking mixtures containing the immobilized hemin and the heme-protein, respectively, were very similar.

TABLE 17

Flavor compounds produced by cooking mixtures containing either myoglobin free in solution or hemin coupled to a solid support		
Flavor compound	myoglobin	hemin-linker-resin
2-methyl-5-(methylthio)-thiophene	Low	
dihydro-5-propyl-2(3H)-furanone	Low	
octane	Low	
pyrrole	Low	Low
methanethiol	Low	Low
2-thiophenecarboxaldehyde	Low	Low
methyl-pyrazine	Low	Low
1-hydroxy-2-propanone	Low	Low
propanal	Low	Low
thiophene	Low	medium
pyridine	Low	Low
2-methyl-furan	Low	medium
oxalic acid, butyl propyl ester	Low	Low
pyrazine	medium	Low
oxalic acid, diallyl ester	medium	medium
2-butenal	medium	large
furfural	medium	medium
nonanal	medium	medium
2-ethyl-furan	medium	Low
ethanol	medium	very large
tert-butanol	medium	
3,3'-dithiobis[2-methyl]-furan	medium	medium
m-aminophenylacetylene	medium	medium
2,5-dihydro-3,4-dimethyl-furan	medium	medium
2-acetylthiazole	medium	medium
cyclohexane	medium	
ethyl tert-butyl ether	medium	
carbon disulfide	medium	medium
thiazole	medium	medium
acetonitrile	medium	large
2-pentyl-furan	medium	Low
3-thiophenecarboxaldehyde	medium	medium
2-methyl-butanal	medium	medium
thiazole	medium	large
2-methyl-3-furanthiol	large	large
2-propenal	large	large
3-methyl-2-butenal	large	medium
2-methyl-3-(methylthio) furan	large	large
ethyl acetate	large	medium
methacrolein	large	medium
methyl-thiirane	large	large
methional	large	large
methyl alcohol	large	medium
2-butanone	large	Low
2,3-butanedione	large	medium
acetone	large	large
furan	large	medium
benzaldehyde	large	medium
methyl thiolacetate	large	medium
acetaldehyde	very large	very large
2-methyl-propanal	very large	very large
dimethyl trisulfide	very large	very large
3-methyl-butanal	very large	very large
propyl-cyclopropane		medium
(E)-2-octenal		medium
2-n-propylaziridine		medium
thiirane		medium
ethyl formate		medium
methyl vinyl ketone		medium
2-propenoic acid, ethyl ester		medium
1-nonanol		large
1-octene		large
1-heptanol		large
1-dodecene		large
phorone		very large

42

Example 14. The Combination of Precursors with Heme Protein Drives Flavor Reactions

Three samples were compared: precursor mix alone, 1% heme protein alone, and precursor mix with 1% heme. The precursor mix was made of glucose (20 mM), ribose (20 mM), cysteine (10 mM), thiamine (1 mM), and glutamic acid (1 mM). Reactions were all at pH 6.0, prepared and heated to 150° C. for 3 minutes. These three samples were run in duplicate. These samples were evaluated by GCMS for the flavor compounds generated. Characteristic flavor and fragrance components were mostly produced during the cooking process where precursors could react with the heme-protein. These samples were evaluated by GCMS for the flavor compounds generated and evaluated for the sensory experience. Volatile chemicals were isolated from the head space around the flavor reaction. The flavor compounds created in each sample is indicated in Table 18. As shown most of the flavor molecules were created on when the precursors are combined with the heme protein.

TABLE 18

Flavor molecules created by the combination of LegH and precursor mix.			
Compound	Precursor mix	LegH	Precursor mix + Leg H
carbon disulfide	medium	medium	high
isopropyl alcohol	medium	medium	low
2-methyl-furan	low		low
butanal	low		medium
thiophene	low		low
2,3-butanedione	low	low	high
furan	low		medium
2,4-dimethyl-1-heptene		high	high
acetone		high	high
dimethyl trisulfide		medium	medium
2-methyl-heptane		medium	medium
2-pentanone		medium	
pentanal		medium	medium
2-pentyl-furan		medium	medium
2-methyl-propanal		low	high
2-acetyl-1-propene		low	low
2-methyl-butanal		low	medium
1,3-dimethyl-benzene		low	low
octane		low	low
benzene		low	low
benzaldehyde			very high
2-butanone			very high
furfural			very high
thiazole			high
nonanal			high
thiazole			high
2-acetylthiazole			medium
3-methyl-butanal			medium
(Z)-2-heptenal			medium
heptanal			medium
methyl-thiirane			medium
3-ethyl-pentane			medium
phenylacetaldehyde			medium
2-hexyl-furan			medium
2-nonanone			medium
propanal			medium
pyrazine			medium
(Z)-2-heptenal			medium
2-methyl-1-heptene			medium
2-ethyl-furan			medium
octanal			medium
(E)-4-octene			low
(E)-2-octenal			low
2-methyl-thiazole			low
2-propenal			low
1-octen-3-one			low
1-octene			low
2-octanone			low

TABLE 18-continued

Flavor molecules created by the combination of LegH and precursor mix.			
Compound	Precursor mix	LegH	Precursor mix + Leg H
dimethyl sulfide			low
3-pentyl-furan			low
2-n-octylfuran			low
2-pentyl-thiophene			low

Other Embodiments

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 27

<210> SEQ ID NO 1

<211> LENGTH: 161

<212> TYPE: PRT

<213> ORGANISM: *Vigna radiata*

<400> SEQUENCE: 1

Met Thr Thr Thr Leu Glu Arg Gly Phe Thr Glu Glu Gln Glu Ala Leu
1 5 10 15

Val Val Lys Ser Trp Asn Val Met Lys Lys Asn Ser Gly Glu Leu Gly
20 25 30

Leu Lys Phe Phe Leu Lys Ile Phe Glu Ile Ala Pro Ser Ala Gln Lys
35 40 45

Leu Phe Ser Phe Leu Arg Asp Ser Thr Val Pro Leu Glu Gln Asn Pro
50 55 60

Lys Leu Lys Pro His Ala Val Ser Val Phe Val Met Thr Cys Asp Ser
65 70 75 80

Ala Val Gln Leu Arg Lys Ala Gly Lys Val Thr Val Arg Glu Ser Asn
85 90 95

Leu Lys Lys Leu Gly Ala Thr His Phe Arg Thr Gly Val Ala Asn Glu
100 105 110

His Phe Glu Val Thr Lys Phe Ala Leu Leu Glu Thr Ile Lys Glu Ala
115 120 125

Val Pro Glu Met Trp Ser Pro Ala Met Lys Asn Ala Trp Gly Glu Ala
130 135 140

Tyr Asp Gln Leu Val Asp Ala Ile Lys Tyr Glu Met Lys Pro Pro Ser
145 150 155 160

Ser

<210> SEQ ID NO 2

<211> LENGTH: 133

<212> TYPE: PRT

<213> ORGANISM: *Methylobacterium thermophilum*

<400> SEQUENCE: 2

Met Ile Asp Gln Lys Glu Lys Glu Leu Ile Lys Glu Ser Trp Lys Arg
1 5 10 15

Ile Glu Pro Asn Lys Asn Glu Ile Gly Leu Leu Phe Tyr Ala Asn Leu
20 25 30

Phe Lys Glu Glu Pro Thr Val Ser Val Leu Phe Gln Asn Pro Ile Ser
35 40 45

Ser Gln Ser Arg Lys Leu Met Gln Val Leu Gly Ile Leu Val Gln Gly
50 55 60

Ile Asp Asn Leu Glu Gly Leu Ile Pro Thr Leu Gln Asp Leu Gly Arg

-continued

65	70	75	80
Arg His Lys Gln Tyr Gly Val Val Asp Ser His Tyr Pro Leu Val Gly			
	85	90	95
Asp Cys Leu Leu Lys Ser Ile Gln Glu Tyr Leu Gly Gln Gly Phe Thr			
	100	105	110
Glu Glu Ala Lys Ala Ala Trp Thr Lys Val Tyr Gly Ile Ala Ala Gln			
	115	120	125
Val Met Thr Ala Glu			
130			

<210> SEQ ID NO 3
 <211> LENGTH: 139
 <212> TYPE: PRT
 <213> ORGANISM: Aquifex aeolicus

<400> SEQUENCE: 3

Met Leu Ser Glu Glu Thr Ile Arg Val Ile Lys Ser Thr Val Pro Leu			
1	5	10	15
Leu Lys Glu His Gly Thr Glu Ile Thr Ala Arg Met Tyr Glu Leu Leu			
	20	25	30
Phe Ser Lys Tyr Pro Lys Thr Lys Glu Leu Phe Ala Gly Ala Ser Glu			
	35	40	45
Glu Gln Pro Lys Lys Leu Ala Asn Ala Ile Ile Ala Tyr Ala Thr Tyr			
	50	55	60
Ile Asp Arg Leu Glu Glu Leu Asp Asn Ala Ile Ser Thr Ile Ala Arg			
65	70	75	80
Ser His Val Arg Arg Asn Val Lys Pro Glu His Tyr Pro Leu Val Lys			
	85	90	95
Glu Cys Leu Leu Gln Ala Ile Glu Glu Val Leu Asn Pro Gly Glu Glu			
	100	105	110
Val Leu Lys Ala Trp Glu Glu Ala Tyr Asp Phe Leu Ala Lys Thr Leu			
	115	120	125
Ile Thr Leu Glu Lys Lys Leu Tyr Ser Gln Pro			
130	135		

<210> SEQ ID NO 4
 <211> LENGTH: 145
 <212> TYPE: PRT
 <213> ORGANISM: Glycine max

<400> SEQUENCE: 4

Met Gly Ala Phe Thr Glu Lys Gln Glu Ala Leu Val Ser Ser Ser Phe			
1	5	10	15
Glu Ala Phe Lys Ala Asn Ile Pro Gln Tyr Ser Val Val Phe Tyr Thr			
	20	25	30
Ser Ile Leu Glu Lys Ala Pro Ala Ala Lys Asp Leu Phe Ser Phe Leu			
	35	40	45
Ser Asn Gly Val Asp Pro Ser Asn Pro Lys Leu Thr Gly His Ala Glu			
	50	55	60
Lys Leu Phe Gly Leu Val Arg Asp Ser Ala Gly Gln Leu Lys Ala Asn			
65	70	75	80
Gly Thr Val Val Ala Asp Ala Ala Leu Gly Ser Ile His Ala Gln Lys			
	85	90	95
Ala Ile Thr Asp Pro Gln Phe Val Val Val Lys Glu Ala Leu Leu Lys			
	100	105	110
Thr Ile Lys Glu Ala Val Gly Asp Lys Trp Ser Asp Glu Leu Ser Ser			

-continued

115	120	125
Ala Trp Glu Val Ala Tyr Asp	Glu Leu Ala Ala	Ala Ile Lys Lys Ala
130	135	140

Phe
145

<210> SEQ ID NO 5
 <211> LENGTH: 162
 <212> TYPE: PRT
 <213> ORGANISM: Hordeum vulgare

<400> SEQUENCE: 5

Met Ser Ala Ala Glu Gly Ala Val Val Phe Ser Glu Glu Lys Glu Ala		
1	5	10 15
Leu Val Leu Lys Ser Trp Ala Ile Met Lys Lys Asp Ser Ala Asn Leu		
	20	25 30
Gly Leu Arg Phe Phe Leu Lys Ile Phe Glu Ile Ala Pro Ser Ala Arg		
	35	40 45
Gln Met Phe Pro Phe Leu Arg Asp Ser Asp Val Pro Leu Glu Thr Asn		
	50	55 60
Pro Lys Leu Lys Thr His Ala Val Ser Val Phe Val Met Thr Cys Glu		
	65	70 75 80
Ala Ala Ala Gln Leu Arg Lys Ala Gly Lys Ile Thr Val Arg Glu Thr		
	85	90 95
Thr Leu Lys Arg Leu Gly Gly Thr His Leu Lys Tyr Gly Val Ala Asp		
	100	105 110
Gly His Phe Glu Val Thr Arg Phe Ala Leu Leu Glu Thr Ile Lys Glu		
	115	120 125
Ala Leu Pro Ala Asp Met Trp Gly Pro Glu Met Arg Asn Ala Trp Gly		
	130	135 140
Glu Ala Tyr Asp Gln Leu Val Ala Ala Ile Lys Gln Glu Met Lys Pro		
	145	150 155 160

Ala Glu

<210> SEQ ID NO 6
 <211> LENGTH: 1153
 <212> TYPE: PRT
 <213> ORGANISM: Magnaporthe oryzae

<400> SEQUENCE: 6

Met Asp Gly Ala Val Arg Leu Asp Trp Thr Gly Leu Asp Leu Thr Gly		
1	5	10 15
His Glu Ile His Asp Gly Val Pro Ile Ala Ser Arg Val Gln Val Met		
	20	25 30
Val Ser Phe Pro Leu Phe Lys Asp Gln His Ile Ile Met Ser Ser Lys		
	35	40 45
Glu Ser Pro Ser Arg Lys Ser Ser Thr Ile Gly Gln Ser Thr Arg Asn		
	50	55 60
Gly Ser Cys Gln Ala Asp Thr Gln Lys Gly Gln Leu Pro Pro Val Gly		
	65	70 75 80
Glu Lys Pro Lys Pro Val Lys Glu Asn Pro Met Lys Lys Leu Lys Glu		
	85	90 95
Met Ser Gln Arg Pro Leu Pro Thr Gln His Gly Asp Gly Thr Tyr Pro		
	100	105 110
Thr Glu Lys Lys Leu Thr Gly Ile Gly Glu Asp Leu Lys His Ile Arg		
	115	120 125

Gly 130	Tyr	Asp	Val	Lys	Thr	Leu 135	Leu	Ala	Met	Val	Lys 140	Ser	Lys	Leu	Lys
Gly 145	Glu	Lys	Leu	Lys	Asp 150	Asp	Lys	Thr	Met	Leu 155	Met	Glu	Arg	Val	Met 160
Gln	Leu	Val	Ala	Arg 165	Leu	Pro	Thr	Glu	Ser 170	Lys	Lys	Arg	Ala	Glu	Leu 175
Thr	Asp	Ser	Leu 180	Ile	Asn	Glu	Leu	Trp 185	Glu	Ser	Leu	Asp 190	His	Pro	Pro
Leu	Asn	Tyr 195	Leu	Gly	Pro	Glu	His 200	Ser	Tyr	Arg	Thr	Pro 205	Asp	Gly	Ser
Tyr	Asn 210	His	Pro	Phe	Asn	Pro 215	Gln	Leu	Gly	Ala	Ala 220	Gly	Ser	Arg	Tyr
Ala 225	Arg	Ser	Val	Ile	Pro 230	Thr	Val	Thr	Pro	Pro 235	Gly	Ala	Leu	Pro	Asp 240
Pro	Gly	Leu	Ile	Phe 245	Asp	Ser	Ile	Met	Gly 250	Arg	Thr	Pro	Asn	Ser	Tyr 255
Arg	Lys	His	Pro 260	Asn	Asn	Val	Ser	Ser 265	Ile	Leu	Trp	Tyr 270	Trp	Ala	Thr
Ile	Ile 275	Ile	His	Asp	Ile	Phe	Trp 280	Thr	Asp	Pro	Arg	Asp 285	Ile	Asn	Thr
Asn	Lys 290	Ser	Ser	Ser	Tyr	Leu 295	Asp	Leu	Ala	Pro	Leu 300	Tyr	Gly	Asn	Ser
Gln 305	Glu	Met	Gln	Asp	Ser 310	Ile	Arg	Thr	Phe	Lys 315	Asp	Gly	Arg	Met	Lys 320
Pro	Asp	Cys	Tyr	Ala 325	Asp	Lys	Arg	Leu	Ala 330	Gly	Met	Pro	Pro	Gly	Val 335
Ser	Val	Leu	Leu 340	Ile	Met	Phe	Asn	Arg 345	Phe	His	Asn	His 350	Val	Ala	Glu
Asn	Leu 355	Ala	Leu	Ile	Asn	Glu	Gly 360	Gly	Arg	Phe	Asn	Lys 365	Pro	Ser	Asp
Leu 370	Leu	Glu	Gly	Glu	Ala	Arg 375	Glu	Ala	Ala	Trp	Lys 380	Lys	Tyr	Asp	Asn
Asp 385	Leu	Phe	Gln	Val	Ala 390	Arg	Leu	Val	Thr	Ser 395	Gly	Leu	Tyr	Ile	Asn 400
Ile	Thr	Leu	Val	Asp 405	Tyr	Val	Arg	Asn	Ile	Val	Asn	Leu	Asn	Arg	Val 415
Asp	Thr	Thr	Trp 420	Thr	Leu	Asp	Pro	Arg 425	Gln	Asp	Ala	Gly 430	Ala	His	Val
Gly	Thr 435	Ala	Asp	Gly	Ala	Glu	Arg 440	Gly	Thr	Gly	Asn	Ala 445	Val	Ser	Ala
Glu 450	Phe	Asn	Leu	Cys	Tyr	Arg 455	Trp	His	Ser	Cys	Ile	Ser 460	Glu	Lys	Asp
Ser 465	Lys	Phe	Val	Glu	Ala 470	Gln	Phe	Gln	Asn	Ile	Phe	Gly 475	Lys	Pro	Ala 480
Ser	Glu	Val	Arg	Pro 485	Asp	Glu	Met	Trp	Lys 490	Gly	Phe	Ala	Lys	Met	Glu 495
Gln	Asn	Thr	Pro 500	Ala	Asp	Pro	Gly	Gln	Arg	Thr	Phe	Gly 510	Gly	Phe	Lys
Arg	Gly 515	Pro	Asp	Gly	Lys	Phe	Asp 520	Asp	Asp	Asp	Leu	Val 525	Arg	Cys	Ile
Ser 530	Glu	Ala	Val	Glu	Asp 535	Val	Ala	Gly	Ala	Phe	Gly 540	Ala	Arg	Asn	Val

Pro 545	Gln	Ala	Met	Lys	Val 550	Val	Glu	Thr	Met	Gly 555	Ile	Ile	Gln	Gly	Arg 560
Lys	Trp	Asn	Val	Ala 565	Gly	Leu	Asn	Glu	Phe	Arg 570	Lys	His	Phe	His 575	Leu
Lys	Pro	Tyr	Ser 580	Thr	Phe	Glu	Asp	Ile 585	Asn	Ser	Asp	Pro	Gly 590	Val	Ala
Glu	Ala	Leu	Arg 595	Arg	Leu	Tyr	Asp 600	His	Pro	Asp	Asn	Val 605	Glu	Leu	Tyr
Pro	Gly 610	Leu	Val	Ala	Glu	Glu 615	Asp	Lys	Gln	Pro	Met 620	Val	Pro	Gly	Val
Gly 625	Ile	Ala	Pro	Thr	Tyr 630	Thr	Ile	Ser	Arg	Val 635	Val	Leu	Ser	Asp	Ala 640
Val	Cys	Leu	Val	Arg 645	Gly	Asp	Arg	Phe	Tyr 650	Thr	Thr	Asp	Phe	Thr 655	Pro
Arg	Asn	Leu	Thr 660	Asn	Trp	Gly	Tyr	Lys 665	Glu	Val	Asp	Tyr	Asp 670	Leu	Ser
Val	Asn	His 675	Gly	Cys	Val	Phe	Tyr 680	Lys	Leu	Phe	Ile	Arg 685	Ala	Phe	Pro
Asn	His 690	Phe	Lys	Gln	Asn	Ser 695	Val	Tyr	Ala	His	Tyr 700	Pro	Met	Val	Val
Pro 705	Ser	Glu	Asn	Lys	Arg 710	Ile	Leu	Glu	Ala	Leu 715	Gly	Arg	Ala	Asp	Leu 720
Phe	Asp	Phe	Glu 725	Ala	Pro	Lys	Tyr	Ile	Pro 730	Pro	Arg	Val	Asn	Ile 735	Thr
Ser	Tyr	Gly 740	Gly	Ala	Glu	Tyr	Ile	Leu 745	Glu	Thr	Gln	Glu	Lys 750	Tyr	Lys
Val	Thr 755	Trp	His	Glu	Gly	Leu	Gly 760	Phe	Leu	Met	Gly	Glu 765	Gly	Gly	Leu
Lys 770	Phe	Met	Leu	Ser	Gly 775	Asp	Asp	Pro	Leu	His	Ala 780	Gln	Gln	Arg	Lys
Cys 785	Met	Ala	Ala	Gln	Leu 790	Tyr	Lys	Asp	Gly	Trp 795	Thr	Glu	Ala	Val	Lys 800
Ala	Phe	Tyr	Ala 805	Gly	Met	Met	Glu	Glu	Leu 810	Leu	Val	Ser	Lys	Ser 815	Tyr
Phe	Leu	Gly 820	Asn	Asn	Lys	His	Arg	His 825	Val	Asp	Ile	Ile	Arg 830	Asp	Val
Gly	Asn 835	Met	Val	His	Val	His	Phe 840	Ala	Ser	Gln	Val	Phe 845	Gly	Leu	Pro
Leu 850	Lys	Thr	Ala	Lys	Asn	Pro 855	Thr	Gly	Val	Phe	Thr 860	Glu	Gln	Glu	Met
Tyr 865	Gly	Ile	Leu	Ala	Ala 870	Ile	Phe	Thr	Thr	Ile 875	Phe	Phe	Asp	Leu	Asp 880
Pro	Ser	Lys	Ser 885	Phe	Pro	Leu	Arg	Thr	Lys 890	Thr	Arg	Glu	Val	Cys 895	Gln
Lys	Leu	Ala	Lys 900	Leu	Val	Glu	Ala	Asn 905	Val	Lys	Leu	Ile	Asn 910	Lys	Ile
Pro	Trp 915	Ser	Arg	Gly	Met	Phe	Val 920	Gly	Lys	Pro	Ala	Lys 925	Asp	Glu	Pro
Leu 930	Ser	Ile	Tyr	Gly	Lys	Thr 935	Met	Ile	Lys	Gly	Leu 940	Lys	Ala	His	Gly
Leu 945	Ser	Asp	Tyr	Asp	Ile 950	Ala	Trp	Ser	His	Val 955	Val	Pro	Thr	Ser	Gly 960
Ala	Met	Val	Pro	Asn	Gln	Ala	Gln	Val	Phe	Ala	Gln	Ala	Val	Asp	Tyr

-continued

965					970					975					
Tyr	Leu	Ser	Pro	Ala	Gly	Met	His	Tyr	Ile	Pro	Glu	Ile	His	Met	Val
			980					985					990		
Ala	Leu	Gln	Pro	Ser	Thr	Pro	Glu	Thr	Asp	Ala	Leu	Leu	Leu	Gly	Tyr
		995					1000					1005			
Ala	Met	Glu	Gly	Ile	Arg	Leu	Ala	Gly	Thr	Phe	Gly	Ser	Tyr	Arg	Glu
	1010					1015					1020				
Ala	Ala	Val	Asp	Asp	Val	Val	Lys	Glu	Asp	Asn	Gly	Arg	Gln	Val	Pro
1025						1030					1035				1040
Val	Lys	Ala	Gly	Asp	Arg	Val	Phe	Val	Ser	Phe	Val	Asp	Ala	Ala	Arg
			1045					1050					1055		
Asp	Pro	Lys	His	Phe	Pro	Asp	Pro	Glu	Val	Val	Asn	Pro	Arg	Arg	Pro
		1060						1065					1070		
Ala	Lys	Lys	Tyr	Ile	His	Tyr	Gly	Val	Gly	Pro	His	Ala	Cys	Leu	Gly
	1075						1080					1085			
Arg	Asp	Ala	Ser	Gln	Ile	Ala	Ile	Thr	Glu	Met	Phe	Arg	Cys	Leu	Phe
	1090					1095					1100				
Arg	Arg	Arg	Asn	Val	Arg	Arg	Val	Pro	Gly	Pro	Gln	Gly	Glu	Leu	Lys
1105						1110					1115				1120
Lys	Val	Pro	Arg	Pro	Gly	Gly	Phe	Tyr	Val	Tyr	Met	Arg	Glu	Asp	Trp
			1125					1130					1135		
Gly	Gly	Leu	Phe	Pro	Phe	Pro	Val	Thr	Met	Arg	Val	Met	Trp	Asp	Asp
		1140					1145					1150			

Glu

<210> SEQ ID NO 7

<211> LENGTH: 530

<212> TYPE: PRT

<213> ORGANISM: Fusarium oxysporum

<400> SEQUENCE: 7

Met	Lys	Gly	Ser	Ala	Thr	Leu	Ala	Phe	Ala	Leu	Val	Gln	Phe	Ser	Ala
1				5					10					15	
Ala	Ser	Gln	Leu	Val	Trp	Pro	Ser	Lys	Trp	Asp	Glu	Val	Glu	Asp	Leu
		20						25					30		
Leu	Tyr	Met	Gln	Gly	Gly	Phe	Asn	Lys	Arg	Gly	Phe	Ala	Asp	Ala	Leu
		35					40					45			
Arg	Thr	Cys	Glu	Phe	Gly	Ser	Asn	Val	Pro	Gly	Thr	Gln	Asn	Thr	Ala
	50					55					60				
Glu	Trp	Leu	Arg	Thr	Ala	Phe	His	Asp	Ala	Ile	Thr	His	Asp	Ala	Lys
65				70						75				80	
Ala	Gly	Thr	Gly	Gly	Leu	Asp	Ala	Ser	Ile	Tyr	Trp	Glu	Ser	Ser	Arg
			85						90					95	
Pro	Glu	Asn	Pro	Gly	Lys	Ala	Phe	Asn	Asn	Thr	Phe	Gly	Phe	Phe	Ser
		100						105					110		
Gly	Phe	His	Asn	Pro	Arg	Ala	Thr	Ala	Ser	Asp	Leu	Thr	Ala	Leu	Gly
		115					120					125			
Thr	Val	Leu	Ala	Val	Gly	Ala	Cys	Asn	Gly	Pro	Arg	Ile	Pro	Phe	Arg
	130					135					140				
Ala	Gly	Arg	Ile	Asp	Ala	Tyr	Lys	Ala	Gly	Pro	Ala	Gly	Val	Pro	Glu
145				150						155					160
Pro	Ser	Thr	Asn	Leu	Lys	Asp	Thr	Phe	Ala	Ala	Phe	Thr	Lys	Ala	Gly
			165					170					175		
Phe	Thr	Lys	Glu	Glu	Met	Thr	Ala	Met	Val	Ala	Cys	Gly	His	Ala	Ile

-continued

180					185					190					
Gly	Gly	Val	His	Ser	Val	Asp	Phe	Pro	Glu	Ile	Val	Gly	Ile	Lys	Ala
	195						200					205			
Asp	Pro	Asn	Asn	Asp	Thr	Asn	Val	Pro	Phe	Gln	Lys	Asp	Val	Ser	Ser
	210					215					220				
Phe	His	Asn	Gly	Ile	Val	Thr	Glu	Tyr	Leu	Ala	Gly	Thr	Ser	Lys	Asn
	225				230					235					240
Pro	Leu	Val	Ala	Ser	Lys	Asn	Ala	Thr	Phe	His	Ser	Asp	Lys	Arg	Ile
				245					250					255	
Phe	Asp	Asn	Asp	Lys	Ala	Thr	Met	Lys	Lys	Leu	Ser	Thr	Lys	Ala	Gly
		260						265					270		
Phe	Asn	Ser	Met	Cys	Ala	Asp	Ile	Leu	Thr	Arg	Met	Ile	Asp	Thr	Val
		275					280					285			
Pro	Lys	Ser	Val	Gln	Leu	Thr	Pro	Val	Leu	Glu	Ala	Tyr	Asp	Val	Arg
	290					295					300				
Pro	Tyr	Ile	Thr	Glu	Leu	Ser	Leu	Asn	Asn	Lys	Asn	Lys	Ile	His	Phe
	305				310					315					320
Thr	Gly	Ser	Val	Arg	Val	Arg	Ile	Thr	Asn	Asn	Ile	Arg	Asp	Asn	Asn
			325					330						335	
Asp	Leu	Ala	Ile	Asn	Leu	Ile	Tyr	Val	Gly	Arg	Asp	Gly	Lys	Lys	Val
		340						345					350		
Thr	Val	Pro	Thr	Gln	Gln	Val	Thr	Phe	Gln	Gly	Gly	Thr	Ser	Phe	Gly
		355					360					365			
Ala	Gly	Glu	Val	Phe	Ala	Asn	Phe	Glu	Phe	Asp	Thr	Thr	Met	Asp	Ala
	370					375					380				
Lys	Asn	Gly	Ile	Thr	Lys	Phe	Phe	Ile	Gln	Glu	Val	Lys	Pro	Ser	Thr
	385				390					395					400
Lys	Ala	Thr	Val	Thr	His	Asp	Asn	Gln	Lys	Thr	Gly	Gly	Tyr	Lys	Val
			405					410						415	
Asp	Asp	Thr	Val	Leu	Tyr	Gln	Leu	Gln	Gln	Ser	Cys	Ala	Val	Leu	Glu
		420					425						430		
Lys	Leu	Pro	Asn	Ala	Pro	Leu	Val	Val	Thr	Ala	Met	Val	Arg	Asp	Ala
	435					440						445			
Arg	Ala	Lys	Asp	Ala	Leu	Thr	Leu	Arg	Val	Ala	His	Lys	Lys	Pro	Val
	450					455					460				
Lys	Gly	Ser	Ile	Val	Pro	Arg	Phe	Gln	Thr	Ala	Ile	Thr	Asn	Phe	Lys
	465				470					475					480
Ala	Thr	Gly	Lys	Lys	Ser	Ser	Gly	Tyr	Thr	Gly	Phe	Gln	Ala	Lys	Thr
			485					490						495	
Met	Phe	Glu	Glu	Gln	Ser	Thr	Tyr	Phe	Asp	Ile	Val	Leu	Gly	Gly	Ser
		500						505					510		
Pro	Ala	Ser	Gly	Val	Gln	Phe	Leu	Thr	Ser	Gln	Ala	Met	Pro	Ser	Gln
		515					520					525			
Cys	Ser														
	530														

<210> SEQ ID NO 8

<211> LENGTH: 358

<212> TYPE: PRT

<213> ORGANISM: Fusarium graminearum

<400> SEQUENCE: 8

Met	Ala	Ser	Ala	Thr	Arg	Gln	Phe	Ala	Arg	Ala	Ala	Thr	Arg	Ala	Thr
1				5				10					15		

-continued

Arg Asn Gly Phe Ala Ile Ala Pro Arg Gln Val Ile Arg Gln Gln Gly
 20 25 30
 Arg Arg Tyr Tyr Ser Ser Glu Pro Ala Gln Lys Ser Ser Ser Ala Trp
 35 40 45
 Ile Trp Leu Thr Gly Ala Ala Val Ala Gly Gly Ala Gly Tyr Tyr Phe
 50 55 60
 Tyr Gly Asn Ser Ala Ser Ser Ala Thr Ala Lys Val Phe Asn Pro Ser
 65 70 75 80
 Lys Glu Asp Tyr Gln Lys Val Tyr Asn Glu Ile Ala Ala Arg Leu Glu
 85 90 95
 Glu Lys Asp Asp Tyr Asp Asp Gly Ser Tyr Gly Pro Val Leu Val Arg
 100 105 110
 Leu Ala Trp His Ala Ser Gly Thr Tyr Asp Lys Glu Thr Gly Thr Gly
 115 120 125
 Gly Ser Asn Gly Ala Thr Met Arg Phe Ala Pro Glu Ser Asp His Gly
 130 135 140
 Ala Asn Ala Gly Leu Ala Ala Ala Arg Asp Phe Leu Gln Pro Val Lys
 145 150 155 160
 Glu Lys Phe Pro Trp Ile Thr Tyr Ser Asp Leu Trp Ile Leu Ala Gly
 165 170 175
 Val Cys Ala Ile Gln Glu Met Leu Gly Pro Ala Ile Pro Tyr Arg Pro
 180 185 190
 Gly Arg Ser Asp Arg Asp Val Ser Gly Cys Thr Pro Asp Gly Arg Leu
 195 200 205
 Pro Asp Ala Ser Lys Arg Gln Asp His Leu Arg Gly Ile Phe Gly Arg
 210 215 220
 Met Gly Phe Asn Asp Gln Glu Ile Val Ala Leu Ser Gly Ala His Ala
 225 230 235 240
 Leu Gly Arg Cys His Thr Asp Arg Ser Gly Tyr Ser Gly Pro Trp Thr
 245 250 255
 Phe Ser Pro Thr Val Leu Thr Asn Asp Tyr Phe Arg Leu Leu Val Glu
 260 265 270
 Glu Lys Trp Gln Trp Lys Lys Trp Asn Gly Pro Ala Gln Tyr Glu Asp
 275 280 285
 Lys Ser Thr Lys Ser Leu Met Met Leu Pro Ser Asp Ile Ala Leu Ile
 290 295 300
 Glu Asp Lys Lys Phe Lys Pro Trp Val Glu Lys Tyr Ala Lys Asp Asn
 305 310 315 320
 Asp Ala Phe Phe Lys Asp Phe Ser Asn Val Val Leu Arg Leu Phe Glu
 325 330 335
 Leu Gly Val Pro Phe Ala Gln Gly Thr Glu Asn Gln Arg Trp Thr Phe
 340 345 350
 Lys Pro Thr His Gln Glu
 355

<210> SEQ ID NO 9

<211> LENGTH: 122

<212> TYPE: PRT

<213> ORGANISM: Chlamydomonas eugametos

<400> SEQUENCE: 9

Met Ser Leu Phe Ala Lys Leu Gly Gly Arg Glu Ala Val Glu Ala Ala
 1 5 10 15

Val Asp Lys Phe Tyr Asn Lys Ile Val Ala Asp Pro Thr Val Ser Thr
 20 25 30

Tyr	Phe	Ser	Asn	Thr	Asp	Met	Lys	Val	Gln	Arg	Ser	Lys	Gln	Phe	Ala
	35						40					45			
Phe	Leu	Ala	Tyr	Ala	Leu	Gly	Gly	Ala	Ser	Glu	Trp	Lys	Gly	Lys	Asp
	50					55					60				
Met	Arg	Thr	Ala	His	Lys	Asp	Leu	Val	Pro	His	Leu	Ser	Asp	Val	His
65					70					75					80
Phe	Gln	Ala	Val	Ala	Arg	His	Leu	Ser	Asp	Thr	Leu	Thr	Glu	Leu	Gly
				85					90					95	
Val	Pro	Pro	Glu	Asp	Ile	Thr	Asp	Ala	Met	Ala	Val	Val	Ala	Ser	Thr
			100					105					110		
Arg	Thr	Glu	Val	Leu	Asn	Met	Pro	Gln	Gln						
		115					120								

```
<210> SEQ ID NO 10
<211> LENGTH: 121
<212> TYPE: PRT
<213> ORGANISM: Tetrahymena pyriformis
```

<400> SEQUENCE: 10

Met	Asn	Lys	Pro	Gln	Thr	Ile	Tyr	Glu	Lys	Leu	Gly	Gly	Glu	Asn	Ala
1				5					10					15	
Met	Lys	Ala	Ala	Val	Pro	Leu	Phe	Tyr	Lys	Lys	Val	Leu	Ala	Asp	Glu
			20					25					30		
Arg	Val	Lys	His	Phe	Phe	Lys	Asn	Thr	Asp	Met	Asp	His	Gln	Thr	Lys
		35					40					45			
Gln	Gln	Thr	Asp	Phe	Leu	Thr	Met	Leu	Leu	Gly	Gly	Pro	Asn	His	Tyr
	50					55					60				
Lys	Gly	Lys	Asn	Met	Thr	Glu	Ala	His	Lys	Gly	Met	Asn	Leu	Gln	Asn
65					70					75					80
Leu	His	Phe	Asp	Ala	Ile	Ile	Glu	Asn	Leu	Ala	Ala	Thr	Leu	Lys	Glu
				85					90					95	
Leu	Gly	Val	Thr	Asp	Ala	Val	Ile	Asn	Glu	Ala	Ala	Lys	Val	Ile	Glu
			100					105					110		
His	Thr	Arg	Lys	Asp	Met	Leu	Gly	Lys							
		115					120								

```
<210> SEQ ID NO 11
<211> LENGTH: 117
<212> TYPE: PRT
<213> ORGANISM: Paramecium caudatum
```

<400> SEQUENCE: 11

Met 1	Ser	Leu	Phe	Glu 5	Gln	Leu	Gly	Gly	Gln 10	Ala	Ala	Val	Gln 15	Ala	Val
Thr	Ala	Gln	Phe 20	Tyr	Ala	Asn	Ile	Gln 25	Ala	Asp	Ala	Thr	Val 30	Ala	Thr
Phe	Phe	Asn	Gly 35	Ile	Asp	Met	Pro 40	Asn	Gln	Thr	Asn	Lys 45	Thr	Ala	Ala
Phe 50	Leu	Cys	Ala	Ala	Leu	Gly 55	Gly	Pro	Asn	Ala	Trp 60	Thr	Gly	Arg	Asn
Leu 65	Lys	Glu	Val	His 70	Ala	Asn	Met	Gly	Val	Ser 75	Asn	Ala	Gln	Phe	Thr 80
Thr	Val	Ile	Gly 85	His	Leu	Arg	Ser	Ala 90	Leu	Thr	Gly	Ala	Gly 95	Val	Ala
Ala	Ala	Leu	Val 100	Glu	Gln	Thr	Val	Ala 105	Val	Ala	Glu	Thr 110	Val	Arg	Gly

-continued

Asp Val Val Thr Val
115

<210> SEQ ID NO 12
<211> LENGTH: 147
<212> TYPE: PRT
<213> ORGANISM: *Aspergillus niger*

<400> SEQUENCE: 12

Met Pro Leu Thr Pro Glu Gln Ile Lys Ile Ile Lys Ala Thr Val Pro
1 5 10 15
Val Leu Gln Glu Tyr Gly Thr Lys Ile Thr Thr Ala Phe Tyr Met Asn
20 25 30
Met Ser Thr Val His Pro Glu Leu Asn Ala Val Phe Asn Thr Ala Asn
35 40 45
Gln Val Lys Gly His Gln Ala Arg Ala Leu Ala Gly Ala Leu Phe Ala
50 55 60
Tyr Ala Ser His Ile Asp Asp Leu Gly Ala Leu Gly Pro Ala Val Glu
65 70 75 80
Leu Ile Cys Asn Lys His Ala Ser Leu Tyr Ile Gln Ala Asp Glu Tyr
85 90 95
Lys Ile Val Gly Lys Tyr Leu Leu Glu Ala Met Lys Glu Val Leu Gly
100 105 110
Asp Ala Cys Thr Asp Asp Ile Leu Asp Ala Trp Gly Ala Ala Tyr Trp
115 120 125
Ala Leu Ala Asp Ile Met Ile Asn Arg Glu Ala Ala Leu Tyr Lys Gln
130 135 140
Ser Gln Gly
145

<210> SEQ ID NO 13
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: *Zea mays*

<400> SEQUENCE: 13

Met Ala Leu Ala Glu Ala Asp Asp Gly Ala Val Val Phe Gly Glu Glu
1 5 10 15
Gln Glu Ala Leu Val Leu Lys Ser Trp Ala Val Met Lys Lys Asp Ala
20 25 30
Ala Asn Leu Gly Leu Arg Phe Phe Leu Lys Val Phe Glu Ile Ala Pro
35 40 45
Ser Ala Glu Gln Met Phe Ser Phe Leu Arg Asp Ser Asp Val Pro Leu
50 55 60
Glu Lys Asn Pro Lys Leu Lys Thr His Ala Met Ser Val Phe Val Met
65 70 75 80
Thr Cys Glu Ala Ala Ala Gln Leu Arg Lys Ala Gly Lys Val Thr Val
85 90 95
Arg Glu Thr Thr Leu Lys Arg Leu Gly Ala Thr His Leu Arg Tyr Gly
100 105 110
Val Ala Asp Gly His Phe Glu Val Thr Gly Phe Ala Leu Leu Glu Thr
115 120 125
Ile Lys Glu Ala Leu Pro Ala Asp Met Trp Ser Leu Glu Met Lys Lys
130 135 140
Ala Trp Ala Glu Ala Tyr Ser Gln Leu Val Ala Ala Ile Lys Arg Glu
145 150 155 160

-continued

Met Lys Pro Asp Ala
165

<210> SEQ ID NO 14
<211> LENGTH: 169
<212> TYPE: PRT
<213> ORGANISM: *Oryza sativa* subsp. *japonica*

<400> SEQUENCE: 14

Met Ala Leu Val Glu Gly Asn Asn Gly Val Ser Gly Gly Ala Val Ser
1 5 10 15
Phe Ser Glu Glu Gln Glu Ala Leu Val Leu Lys Ser Trp Ala Ile Met
20 25 30
Lys Lys Asp Ser Ala Asn Ile Gly Leu Arg Phe Phe Leu Lys Ile Phe
35 40 45
Glu Val Ala Pro Ser Ala Ser Gln Met Phe Ser Phe Leu Arg Asn Ser
50 55 60
Asp Val Pro Leu Glu Lys Asn Pro Lys Leu Lys Thr His Ala Met Ser
65 70 75 80
Val Phe Val Met Thr Cys Glu Ala Ala Ala Gln Leu Arg Lys Ala Gly
85 90 95
Lys Val Thr Val Arg Asp Thr Thr Leu Lys Arg Leu Gly Ala Thr His
100 105 110
Phe Lys Tyr Gly Val Gly Asp Ala His Phe Glu Val Thr Arg Phe Ala
115 120 125
Leu Leu Glu Thr Ile Lys Glu Ala Val Pro Val Asp Met Trp Ser Pro
130 135 140
Ala Met Lys Ser Ala Trp Ser Glu Ala Tyr Asn Gln Leu Val Ala Ala
145 150 155 160
Ile Lys Gln Glu Met Lys Pro Ala Glu
165

<210> SEQ ID NO 15
<211> LENGTH: 160
<212> TYPE: PRT
<213> ORGANISM: *Arabidopsis thaliana*

<400> SEQUENCE: 15

Met Glu Ser Glu Gly Lys Ile Val Phe Thr Glu Glu Gln Glu Ala Leu
1 5 10 15
Val Val Lys Ser Trp Ser Val Met Lys Lys Asn Ser Ala Glu Leu Gly
20 25 30
Leu Lys Leu Phe Ile Lys Ile Phe Glu Ile Ala Pro Thr Thr Lys Lys
35 40 45
Met Phe Ser Phe Leu Arg Asp Ser Pro Ile Pro Ala Glu Gln Asn Pro
50 55 60
Lys Leu Lys Pro His Ala Met Ser Val Phe Val Met Cys Cys Glu Ser
65 70 75 80
Ala Val Gln Leu Arg Lys Thr Gly Lys Val Thr Val Arg Glu Thr Thr
85 90 95
Leu Lys Arg Leu Gly Ala Ser His Ser Lys Tyr Gly Val Val Asp Glu
100 105 110
His Phe Glu Val Ala Lys Tyr Ala Leu Leu Glu Thr Ile Lys Glu Ala
115 120 125
Val Pro Glu Met Trp Ser Pro Glu Met Lys Val Ala Trp Gly Gln Ala
130 135 140

-continued

Tyr Asp His Leu Val Ala Ala Ile Lys Ala Glu Met Asn Leu Ser Asn
145 150 155 160

<210> SEQ ID NO 16
<211> LENGTH: 147
<212> TYPE: PRT
<213> ORGANISM: Pisum sativum

<400> SEQUENCE: 16

Met Gly Phe Thr Asp Lys Gln Glu Ala Leu Val Asn Ser Ser Trp Glu
1 5 10 15
Ser Phe Lys Gln Asn Leu Ser Gly Asn Ser Ile Leu Phe Tyr Thr Ile
20 25 30
Ile Leu Glu Lys Ala Pro Ala Ala Lys Gly Leu Phe Ser Phe Leu Lys
35 40 45
Asp Thr Ala Gly Val Glu Asp Ser Pro Lys Leu Gln Ala His Ala Glu
50 55 60
Gln Val Phe Gly Leu Val Arg Asp Ser Ala Ala Gln Leu Arg Thr Lys
65 70 75 80
Gly Glu Val Val Leu Gly Asn Ala Thr Leu Gly Ala Ile His Val Gln
85 90 95
Arg Gly Val Thr Asp Pro His Phe Val Val Val Lys Glu Ala Leu Leu
100 105 110
Gln Thr Ile Lys Lys Ala Ser Gly Asn Asn Trp Ser Glu Glu Leu Asn
115 120 125
Thr Ala Trp Glu Val Ala Tyr Asp Gly Leu Ala Thr Ala Ile Lys Lys
130 135 140
Ala Met Thr
145

<210> SEQ ID NO 17
<211> LENGTH: 145
<212> TYPE: PRT
<213> ORGANISM: Vigna unguiculata

<400> SEQUENCE: 17

Met Val Ala Phe Ser Asp Lys Gln Glu Ala Leu Val Asn Gly Ala Tyr
1 5 10 15
Glu Ala Phe Lys Ala Asn Ile Pro Lys Tyr Ser Val Val Phe Tyr Thr
20 25 30
Thr Ile Leu Glu Lys Ala Pro Ala Ala Lys Asn Leu Phe Ser Phe Leu
35 40 45
Ala Asn Gly Val Asp Ala Thr Asn Pro Lys Leu Thr Gly His Ala Glu
50 55 60
Lys Leu Phe Gly Leu Val Arg Asp Ser Ala Ala Gln Leu Arg Ala Ser
65 70 75 80
Gly Gly Val Val Ala Asp Ala Ala Leu Gly Ala Val His Ser Gln Lys
85 90 95
Ala Val Asn Asp Ala Gln Phe Val Val Val Lys Glu Ala Leu Val Lys
100 105 110
Thr Leu Lys Glu Ala Val Gly Asp Lys Trp Ser Asp Glu Leu Gly Thr
115 120 125
Ala Val Glu Leu Ala Tyr Asp Glu Leu Ala Ala Ala Ile Lys Lys Ala
130 135 140
Tyr
145

-continued

<210> SEQ ID NO 18
 <211> LENGTH: 154
 <212> TYPE: PRT
 <213> ORGANISM: Bos taurus

<400> SEQUENCE: 18

```

Met Gly Leu Ser Asp Gly Glu Trp Gln Leu Val Leu Asn Ala Trp Gly
 1             5             10             15
Lys Val Glu Ala Asp Val Ala Gly His Gly Gln Glu Val Leu Ile Arg
      20             25             30
Leu Phe Thr Gly His Pro Glu Thr Leu Glu Lys Phe Asp Lys Phe Lys
      35             40             45
His Leu Lys Thr Glu Ala Glu Met Lys Ala Ser Glu Asp Leu Lys Lys
      50             55             60
His Gly Asn Thr Val Leu Thr Ala Leu Gly Gly Ile Leu Lys Lys Lys
      65             70             75             80
Gly His His Glu Ala Glu Val Lys His Leu Ala Glu Ser His Ala Asn
      85             90             95
Lys His Lys Ile Pro Val Lys Tyr Leu Glu Phe Ile Ser Asp Ala Ile
      100            105            110
Ile His Val Leu His Ala Lys His Pro Ser Asp Phe Gly Ala Asp Ala
      115            120            125
Gln Ala Ala Met Ser Lys Ala Leu Glu Leu Phe Arg Asn Asp Met Ala
      130            135            140
Ala Gln Tyr Lys Val Leu Gly Phe His Gly
      145            150

```

<210> SEQ ID NO 19
 <211> LENGTH: 154
 <212> TYPE: PRT
 <213> ORGANISM: Sus scrofa

<400> SEQUENCE: 19

```

Met Gly Leu Ser Asp Gly Glu Trp Gln Leu Val Leu Asn Val Trp Gly
 1             5             10             15
Lys Val Glu Ala Asp Val Ala Gly His Gly Gln Glu Val Leu Ile Arg
      20             25             30
Leu Phe Lys Gly His Pro Glu Thr Leu Glu Lys Phe Asp Lys Phe Lys
      35             40             45
His Leu Lys Ser Glu Asp Glu Met Lys Ala Ser Glu Asp Leu Lys Lys
      50             55             60
His Gly Asn Thr Val Leu Thr Ala Leu Gly Gly Ile Leu Lys Lys Lys
      65             70             75             80
Gly His His Glu Ala Glu Leu Thr Pro Leu Ala Gln Ser His Ala Thr
      85             90             95
Lys His Lys Ile Pro Val Lys Tyr Leu Glu Phe Ile Ser Glu Ala Ile
      100            105            110
Ile Gln Val Leu Gln Ser Lys His Pro Gly Asp Phe Gly Ala Asp Ala
      115            120            125
Gln Gly Ala Met Ser Lys Ala Leu Glu Leu Phe Arg Asn Asp Met Ala
      130            135            140
Ala Lys Tyr Lys Glu Leu Gly Phe Gln Gly
      145            150

```

<210> SEQ ID NO 20

-continued

```

<211> LENGTH: 154
<212> TYPE: PRT
<213> ORGANISM: Equus caballus

<400> SEQUENCE: 20

Met Gly Leu Ser Asp Gly Glu Trp Gln Gln Val Leu Asn Val Trp Gly
 1             5             10             15

Lys Val Glu Ala Asp Ile Ala Gly His Gly Gln Glu Val Leu Ile Arg
      20             25             30

Leu Phe Thr Gly His Pro Glu Thr Leu Glu Lys Phe Asp Lys Phe Lys
      35             40             45

His Leu Lys Thr Glu Ala Glu Met Lys Ala Ser Glu Asp Leu Lys Lys
      50             55             60

His Gly Thr Val Val Leu Thr Ala Leu Gly Gly Ile Leu Lys Lys Lys
      65             70             75             80

Gly His His Glu Ala Glu Leu Lys Pro Leu Ala Gln Ser His Ala Thr
      85             90             95

Lys His Lys Ile Pro Ile Lys Tyr Leu Glu Phe Ile Ser Asp Ala Ile
      100            105            110

Ile His Val Leu His Ser Lys His Pro Gly Asp Phe Gly Ala Asp Ala
      115            120            125

Gln Gly Ala Met Thr Lys Ala Leu Glu Leu Phe Arg Asn Asp Ile Ala
      130            135            140

Ala Lys Tyr Lys Glu Leu Gly Phe Gln Gly
145            150

```

```

<210> SEQ ID NO 21
<211> LENGTH: 152
<212> TYPE: PRT
<213> ORGANISM: Nicotiana benthamiana

<400> SEQUENCE: 21

Met Ser Ser Phe Thr Glu Glu Gln Glu Ala Leu Val Val Lys Ser Trp
 1             5             10             15

Asp Ser Met Lys Lys Asn Ala Gly Glu Trp Gly Leu Lys Leu Phe Leu
      20             25             30

Lys Ile Phe Glu Ile Ala Pro Ser Ala Lys Lys Leu Phe Ser Phe Leu
      35             40             45

Lys Asp Ser Asn Val Pro Leu Glu Gln Asn Ala Lys Leu Lys Pro His
      50             55             60

Ser Lys Ser Val Phe Val Met Thr Cys Glu Ala Val Gln Leu Arg
      65             70             75             80

Lys Ala Gly Lys Val Val Val Arg Asp Ser Thr Leu Lys Lys Leu Gly
      85             90             95

Ala Thr His Phe Lys Tyr Gly Val Ala Asp Glu His Phe Glu Val Thr
      100            105            110

Lys Phe Ala Leu Leu Glu Thr Ile Lys Glu Ala Val Pro Glu Met Trp
      115            120            125

Ser Val Asp Met Lys Asn Ala Trp Gly Glu Ala Phe Asp Gln Leu Val
      130            135            140

Asn Ala Ile Lys Thr Glu Met Lys
145            150

```

```

<210> SEQ ID NO 22
<211> LENGTH: 132
<212> TYPE: PRT
<213> ORGANISM: Bacillus subtilis

```

-continued

<400> SEQUENCE: 22

```

Met Gly Gln Ser Phe Asn Ala Pro Tyr Glu Ala Ile Gly Glu Glu Leu
 1           5           10           15
Leu Ser Gln Leu Val Asp Thr Phe Tyr Glu Arg Val Ala Ser His Pro
 20           25           30
Leu Leu Lys Pro Ile Phe Pro Ser Asp Leu Thr Glu Thr Ala Arg Lys
 35           40           45
Gln Lys Gln Phe Leu Thr Gln Tyr Leu Gly Gly Pro Pro Leu Tyr Thr
 50           55           60
Glu Glu His Gly His Pro Met Leu Arg Ala Arg His Leu Pro Phe Pro
 65           70           75           80
Ile Thr Asn Glu Arg Ala Asp Ala Trp Leu Ser Cys Met Lys Asp Ala
 85           90           95
Met Asp His Val Gly Leu Glu Gly Glu Ile Arg Glu Phe Leu Phe Gly
 100          105          110
Arg Leu Glu Leu Thr Ala Arg His Met Val Asn Gln Thr Glu Ala Glu
 115          120          125
Asp Arg Ser Ser
 130

```

<210> SEQ ID NO 23

<211> LENGTH: 131

<212> TYPE: PRT

<213> ORGANISM: *Corynebacterium glutamicum*

<400> SEQUENCE: 23

```

Met Thr Thr Ser Glu Asn Phe Tyr Asp Ser Val Gly Gly Glu Glu Thr
 1           5           10           15
Phe Ser Leu Ile Val His Arg Phe Tyr Glu Gln Val Pro Asn Asp Asp
 20           25           30
Ile Leu Gly Pro Met Tyr Pro Pro Asp Asp Phe Glu Gly Ala Glu Gln
 35           40           45
Arg Leu Lys Met Phe Leu Ser Gln Tyr Trp Gly Gly Pro Lys Asp Tyr
 50           55           60
Gln Glu Gln Arg Gly His Pro Arg Leu Arg Met Arg His Val Asn Tyr
 65           70           75           80
Pro Ile Gly Val Thr Ala Ala Glu Arg Trp Leu Gln Leu Met Ser Asn
 85           90           95
Ala Leu Asp Gly Val Asp Leu Thr Ala Glu Gln Arg Glu Ala Ile Trp
 100          105          110
Glu His Met Val Arg Ala Ala Asp Met Leu Ile Asn Ser Asn Pro Asp
 115          120          125
Pro His Ala
 130

```

<210> SEQ ID NO 24

<211> LENGTH: 124

<212> TYPE: PRT

<213> ORGANISM: *Synechocystis* sp.

<400> SEQUENCE: 24

```

Met Ser Thr Leu Tyr Glu Lys Leu Gly Gly Thr Thr Ala Val Asp Leu
 1           5           10           15
Ala Val Asp Lys Phe Tyr Glu Arg Val Leu Gln Asp Asp Arg Ile Lys
 20           25           30

```

-continued

His Phe Phe Ala Asp Val Asp Met Ala Lys Gln Arg Ala His Gln Lys
 35 40 45

Ala Phe Leu Thr Tyr Ala Phe Gly Gly Thr Asp Lys Tyr Asp Gly Arg
 50 55 60

Tyr Met Arg Glu Ala His Lys Glu Leu Val Glu Asn His Gly Leu Asn
 65 70 75 80

Gly Glu His Phe Asp Ala Val Ala Glu Asp Leu Leu Ala Thr Leu Lys
 85 90 95

Glu Met Gly Val Pro Glu Asp Leu Ile Ala Glu Val Ala Ala Val Ala
 100 105 110

Gly Ala Pro Ala His Lys Arg Asp Val Leu Asn Gln
 115 120

<210> SEQ ID NO 25
 <211> LENGTH: 183
 <212> TYPE: PRT
 <213> ORGANISM: Synechococcus sp.

<400> SEQUENCE: 25

Met Asp Val Ala Leu Leu Glu Lys Ser Phe Glu Gln Ile Ser Pro Arg
 1 5 10 15

Ala Ile Glu Phe Ser Ala Ser Phe Tyr Gln Asn Leu Phe His His His
 20 25 30

Pro Glu Leu Lys Pro Leu Phe Ala Glu Thr Ser Gln Thr Ile Gln Glu
 35 40 45

Lys Lys Leu Ile Phe Ser Leu Ala Ala Ile Ile Glu Asn Leu Arg Asn
 50 55 60

Pro Asp Ile Leu Gln Pro Ala Leu Lys Ser Leu Gly Ala Arg His Ala
 65 70 75 80

Glu Val Gly Thr Ile Lys Ser His Tyr Pro Leu Val Gly Gln Ala Leu
 85 90 95

Ile Glu Thr Phe Ala Glu Tyr Leu Ala Ala Asp Trp Thr Glu Gln Leu
 100 105 110

Ala Thr Ala Trp Val Glu Ala Tyr Asp Val Ile Ala Ser Thr Met Ile
 115 120 125

Glu Gly Ala Asp Asn Pro Ala Ala Tyr Leu Glu Pro Glu Leu Thr Phe
 130 135 140

Tyr Glu Trp Leu Asp Leu Tyr Gly Glu Glu Ser Pro Lys Val Arg Asn
 145 150 155 160

Ala Ile Ala Thr Leu Thr His Phe His Tyr Gly Glu Asp Pro Gln Asp
 165 170 175

Val Gln Arg Asp Ser Arg Gly
 180

<210> SEQ ID NO 26
 <211> LENGTH: 118
 <212> TYPE: PRT
 <213> ORGANISM: Nostoc commune

<400> SEQUENCE: 26

Met Ser Thr Leu Tyr Asp Asn Ile Gly Gly Gln Pro Ala Ile Glu Gln
 1 5 10 15

Val Val Asp Glu Leu His Lys Arg Ile Ala Thr Asp Ser Leu Leu Ala
 20 25 30

Pro Val Phe Ala Gly Thr Asp Met Val Lys Gln Arg Asn His Leu Val
 35 40 45

-continued

```

Ala Phe Leu Ala Gln Ile Phe Glu Gly Pro Lys Gln Tyr Gly Gly Arg
 50          55          60

Pro Met Asp Lys Thr His Ala Gly Leu Asn Leu Gln Gln Pro His Phe
65          70          75          80

Asp Ala Ile Ala Lys His Leu Gly Glu Arg Met Ala Val Arg Gly Val
          85          90          95

Ser Ala Glu Asn Thr Lys Ala Ala Leu Asp Arg Val Thr Asn Met Lys
          100         105         110

Gly Ala Ile Leu Asn Lys
          115

```

```

<210> SEQ ID NO 27
<211> LENGTH: 136
<212> TYPE: PRT
<213> ORGANISM: Bacillus megaterium

<400> SEQUENCE: 27

```

```

Met Arg Glu Lys Ile His Ser Pro Tyr Glu Leu Leu Gly Gly Glu His
 1          5          10          15

Thr Ile Ser Lys Leu Val Asp Ala Phe Tyr Thr Arg Val Gly Gln His
          20          25          30

Pro Glu Leu Ala Pro Ile Phe Pro Asp Asn Leu Thr Glu Thr Ala Arg
          35          40          45

Lys Gln Lys Gln Phe Leu Thr Gln Tyr Leu Gly Gly Pro Ser Leu Tyr
          50          55          60

Thr Glu Glu His Gly His Pro Met Leu Arg Ala Arg His Leu Pro Phe
65          70          75          80

Glu Ile Thr Pro Ser Arg Ala Lys Ala Trp Leu Thr Cys Met His Glu
          85          90          95

Ala Met Asp Glu Ile Asn Leu Glu Gly Pro Glu Arg Asp Glu Leu Tyr
          100         105         110

His Arg Leu Ile Leu Thr Ala Gln His Met Ile Asn Ser Pro Glu Gln
          115         120         125

Thr Asp Glu Lys Gly Phe Ser His
          130         135

```

What is claimed is:

1. A ground beef-like food product comprising:

- a) 0.1%-5% by weight of a heme-containing protein comprising an amino acid sequence having at least 80% sequence identity to the polypeptide set forth in SEQ ID NO:4;
- b) a compound selected from glucose, ribose, fructose, lactose, xylose, arabinose, glucose-6-phosphate, maltose, and galactose, and mixtures of two or more thereof;
- c) at least 10 mM of a compound selected from cysteine, cystine, selenocysteine, thiamine, methionine, and mixtures of two or more thereof; and

d) 10% or more by weight of one or more plant proteins, wherein the ground beef-like food product contains no animal products, and wherein cooking the ground beef-like food product results in the production of at least two volatile compounds which have a beef-associated aroma.

2. The ground beef-like food product of claim 1, wherein the heme-containing protein comprises an amino acid sequence having at least 85% sequence identity to the polypeptide set forth in SEQ ID NO:4.

3. The ground beef-like food product of claim 1, wherein the heme-containing protein comprises an amino acid sequence having at least 90% sequence identity to the polypeptide set forth in SEQ ID NO:4.

4. The ground beef-like food product of claim 1, wherein the heme-containing protein comprises an amino acid sequence having at least 95% sequence identity to the polypeptide set forth in SEQ ID NO:4.

5. The ground beef-like food product of claim 1, wherein the heme-containing protein comprises a polypeptide as set forth in SEQ ID NO:4.

6. The ground beef-like food product of claim 1, further comprising one or more of inosine, inosine monophosphate (IMP), guanosine, guanosine monophosphate (GMP), and adenosine monophosphate (AMP).

7. The ground beef-like food product of claim 1, further comprising one or more of beta-carotene, alpha-tocopherol, caffeic acid, propyl gallate, and epigallocatechin gallate.

8. The ground beef-like food product of claim 1, further comprising one or more of a vegetable oil, an algal oil, sunflower oil, corn oil, soybean oil, palm fruit oil, palm kernel oil, safflower oil, flaxseed oil, rice bran oil, cottonseed oil, olive oil, canola oil, flaxseed oil, coconut oil, and mango oil.

77

9. The ground beef-like food product of claim 1, further comprising coconut oil.

10. The ground beef-like food product of claim 1, further comprising lactic acid.

11. The ground beef-like food product of claim 1, comprising a textured vegetable protein.

12. The ground beef-like food product of claim 1, wherein the ground beef-like food product has a pink to red color before cooking to indicate a raw or uncooked state.

13. The ground beef-like food product of claim 1, wherein at least a portion of the ground beef-like food product, upon cooking, transitions in color from a pink to red color in a raw or uncooked state to a lighter pink to brown color in a partially cooked to fully cooked state.

14. The ground beef-like food product of claim 1, comprising about 5.6 to about 20 mM of the compound selected from glucose, ribose, fructose, lactose, xylose, arabinose, glucose-6-phosphate, maltose, and galactose, and mixtures of two or more thereof.

15. The ground beef-like food product of claim 1, comprising about 0.8 mM to about 10 mM cysteine.

16. The ground beef-like food product of claim 1, comprising about 0.1 mM to about 2 mM thiamine.

17. The ground beef-like food product of claim 1, wherein the at least two volatile compounds are selected from 2-methyl-furan, bis(2-methyl-3-furyl)disulfide, 2-pentyl-furan, 3,3'-dithiobis-2-methyl-furan, 2,5-dimethyl-pyrazine, 2-methyl-3-furanthiol, dihydro-3-(2H)-thiophenone, 5-methyl-2-thiophenecarboxaldehyde, 3-methyl-2-thiophenecarboxaldehyde, 2-methyl-thiazole, dimethyl sulfide,

78

decanal, 5-ethylidihydro-2(3H)-furanone, dihydro-5-pentyl-2(3H)-furanone, 2-octanone, 3,5-octadien-2-one, p-Cresol, and hexanoic acid.

18. The ground beef-like food product of claim 1, wherein cooking comprises heating the ground beef-like food product at 150° C. for about 3 to about 5 minutes.

19. The ground beef-like food product of claim 1, further comprising one or more of acetic acid, lactic acid, glycolic acid, citric acid, succinic acid, tartaric acid, caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid, alpha linolenic acid, gamma linolenic acid, arachidic acid, arachidonic acid, behenic acid, and erucic acid.

20. The ground beef-like food product of claim 1, wherein cooking the ground beef-like food product results in the production of at least five volatile compounds which have a beef-associated aroma.

21. The ground beef-like food product of claim 1, wherein cooking the ground beef-like food product results in the production of at least ten volatile compounds which have a beef-associated aroma.

22. The ground beef-like food product of claim 1, wherein cooking the ground beef-like food product results in the production of at least twenty volatile compounds which have a beef-associated aroma.

23. The ground beef-like food product of claim 1, wherein the at least two volatile compounds are 2-methyl-furan and bis(2-methyl-3-furyl)disulfide.

* * * * *